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MEASUREMENT OF THE THICKNESS OF THE BRONCHIAL EPITHELIUM

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MEASUREMENT OF THICKNESS OF BRONCHIAL EPITHELIUM

A report by Drummond H. Bowden, M.D., and Frank Baldwin, Ph.D., under contract to the Atomic Energy Control Board.

ABSTRACT

Cancer of the lung in uranium miners is thought to be related to the inhalation of gaseous radon daughters which become attached to molecules of water vapour or to dust particles.

Since the depth of tissue penetration by alpha particles is short, the thickness of the epithelium that lines the bronchial tree may be a critical factor in the development of cancers at specific sites in the lung. The objectives of the present study were:

1. to measure the thickness of human bronchial epithelium,
2. to determine the distribution and depth of the nuclei of basal cells in the bronchial epithelium, and
3. to compare these parameters in groups of smokers and non-smokers.

Twenty-nine surgically removed specimens of the lung were examined: 26 smokers, 3 non-smokers. The specimens were fixed and prepared for examination by light and electron microscopy. Blocks of tissue were oriented so that the maximum number of bronchi were cut in cross-section; measurements included bronchi of all sizes from bronchial generation 1 ($\geq$ 9.01 mm) diameter to the smallest bronchioles, generations 7 - 16 (0.26 - 2.0 mm).

Comparison of measurements in smokers and non-smokers show no significant differences, so that the 29 cases are considered to represent a homogeneous group.

With progressive divisions of the bronchi, the epithelium decreases in thickness. Of more importance are the figures relating to the distance from the cell surface to the underlying nucleus. Here too, with the exception of goblet cells, the measurements are significantly smaller in generations 7 - 16 than in generation 1. Allowing for shrinkage of tissue during preparation, we arrive at the following figures :

AVERAGE MEASUREMENTS

Cell Type	Distance of Nucleus from Mucociliary Surface (µm)	
	Generation 1	Generations 7 - 16
Basal cells (typical)	38	28
Basal cells (atypical)	34	27
Goblet cells	31	29
Ciliated cells	24	18
Non-ciliated cells	21	17

Thus, the overall range of means for all epithelial cells throughout the bronchial tree is 17 - 38 µm. These figures may be used to calculate the accessibility of bronchial epithelial nuclei to the inhaled products of uranium decay.

RÉSUMÉ

On pense que le cancer du poumon chez les mineurs d'uranium est associé à l'aspiration des produits de filiation gazeux du radon qui s'attachent aux molécules de vapeur d'eau et aux poussières.

Comme les particules alpha pénètrent peu profondément dans les tissus, l'épaisseur de l'épithélium qui tapisse l'arbre bronchique pourrait représenter un facteur critique dans le développement de tumeurs cancéreuses dans des endroits précis du poumon.

La présente étude a pour but de :

1. mesurer l'épaisseur de l'épithélium bronchique humain;
2. déterminer la distribution et la profondeur du noyau des cellules basales dans l'épithélium bronchique;
3. comparer ces paramètres dans des groupes de fumeurs et de non-fumeurs.

Vingt-neuf spécimens de poumons de 26 fumeurs et de 3 non-fumeurs ont été prélevés chirurgicalement et examinés. Les spécimens ont été conservés et préparés pour l'examen par microscopie électronique et ordinaire. Les blocs de tissus ont été orientés de façon que le maximum de bronches soit coupé transversalement. Les mesures comprenaient les bronches de toutes dimensions de la première génération ($\geq$ 9,01 mm) de diamètre aux bronchioles des générations 7 à 16 (0,26 à 2,0 mm).

La comparaison des mesures chez les fumeurs et les non-fumeurs ne fait voir aucune différence significative, de sorte que les 29 cas sont considérés comme représentatifs d'un groupe homogène.

Avec les divisions progressives des bronches, l'épithélium diminue d'épaisseur. Plus importante est la distance entre la surface de la cellule et son noyau. Ici aussi, à l'exception des cellules caliciformes, les mesures sont beaucoup moins importantes dans les générations 7 à 16 que dans la première génération. Compte tenu du rétrécissement des tissus durant la préparation, voici les mesures moyennes :

MESURES MOYENNES

Type de cellules	**Distance du noyau à la surface mucociliaire(µm)**	
	Génération 1	Générations 7 à 16
Cellules basales (typiques)	38	28
Cellules basales (atypiques)	34	27
Cellules caliciformes	31	29
Cellules ciliaires	24	18
Cellules non ciliaires	21	17

Ainsi, la variation de la distance moyenne de toutes les cellules de l'épithélium à travers l'arbre bronchique est de 17 à 38 µm. Ces chiffres peuvent être utilisés pour calculer l'accessibilité des produits de décroissance de l'uranium aspirés aux noyaux des cellules de l'épithélium bronchique.

DISCLAIMER

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A. INTRODUCTION

1. Background

The problem of lung cancer in uranium miners is of some standing. Not only is the prevalence of lung cancer increased, but an unusually high proportion of the very malignant small cell carcinoma is found in the early stages of epidemiological followup of uranium miners. As with some other occupationally related cancers of the lung, smoking exerts a greater than additive effect (BEIR III, 1980; Sevc et al, 1988) and there might even be a multiplicative interaction between the effects of inhaled radioactive materials and cigarette smoking (ICRP, 1987; BEIR IV, 1988).

Carcinogenesis in uranium miners is thought to be related, in a large part, to uranium decay with the production of radon daughters which can be inhaled. Although the decay products are single ionized atoms, they attach to molecules of water vapour or to dust particles as "cluster ions". The median radioactive diameter of these aerosols is 0.03 - 0.15μm, and when they are inhaled they can reach all parts of the lung; some of them (approximately 30%) are retained in the lung. Since the depth of tissue penetration by alpha particles is short and, since the predominant population of dividing cells which are thought to be the most likely source of radiation-induced lung cancer, is in the basal layer, the thickness of the epithelial lining at all levels of the bronchial tree may be a critical factor in the development of neoplastic change at specific sites in the lung. Furthermore, whether their radioactive half-life is short or long, since inhaled radioactive aerosols are probably handled by pulmonary clearance mechanisms in the same manner as other non-radioactive aerosols, the usual patterns of deposition and accumulation in the lung are relevant to an understanding of the induction of cancer in uranium miners. Investigation of these matters should include, therefore, not only the thickness of the epithelium but the location of peri-bronchial deposits of dust and the relationship of these deposits to the bronchial basal cells.

2. Microscopic Anatomy of the Bronchial Tree

The airways are lined by pseudo-stratified epithelium; all of the cells reach the basement membrane (Figure 1). Ciliated columnar, mucus secreting, non-ciliated columnar and basal cells are the preponderant populations; smaller numbers of additional cell types have also been described. The proportions of cells vary with the level of the airway. (Reid and Jones, 1979). The bronchial cells constitute a labile population; hyperplasia of mucous cells is the acute response to irritants (Reid and Jones, 1979) and chronic irritation by tobacco smoke is associated with squamous metaplasia. This change and the accompanying loss of cilia and mucous cells may facilitate epithelial penetration by fibres and particles (Woodworth et al, 1983).

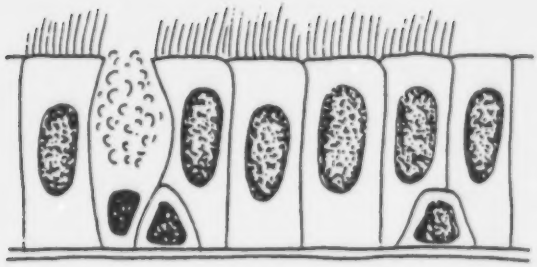


FIGURE 1: Diagram of pseudostratified epithelium showing the cell types measured.

In a section of bronchus cut at a right angle to the surface, a stratified appearance is evident because nuclei are at different levels.

All cells are in contact with the basement membrane (BM), however, only ciliated (C) and non-ciliated columnar cells (NC) and goblet cells (G) reach the surface.

The cytokinetics of airways' epithelium is incompletely understood, and most of the available data pertains to animals. In the mouse, bronchial epithelium turns over in from two to seven days (Bowden, 1983). The role of the basal cell in the renewal of lining cells is of particular interest. For many years, the basal cells have been regarded as the progenitors of other bronchial epithelial cells (Spencer, 1985; Ayers and Jeffrey, 1982). This view is no longer acceptable because secretory cells of the bronchial epithelium can divide and replicate rapidly in response to acute injury (Evans et al, 1986; Keenan et al, 1982a, 1982b). A more recent paper indicates that, notwithstanding the regenerative capacity of secretory cells, basal cells are the real stem cells since, at least in the trachea, they can give rise to all types of epithelium. (Inayama et al, 1988). It has been widely held that proliferation of basal cells is of paramount importance in the development of lung cancer (Auerbach, 1980), however, the role of other proliferative cells in the bronchial epithelium as progenitor of lung cancer remains unresolved.

Although the literature is replete with descriptions of cell types and numbers, only one paper describing the thickness of bronchial epithelium at different levels has been identified (Gastineau et al, 1972). The figures given in this paper suggest

that the basal cell nuclei may be within range of alpha particles impinging upon the surface of the airways' cells. The effects of cigarette smoking were not studied.

3. The Mucus Layer of the Airways

The mucus layer has two components, the sol layer in which the cilia beat and the viscous layer which lies on top of the cilia (Trembath, 1981). For normal ciliary function it has been suggested that an appropriate functional depth of the sol layer would be about 5 μm (Kilburn, 1968), however, the mucus is not distributed uniformly and there is some doubt as to whether the viscous layer is continuous or discontinuous. No reliable measurements of the thickness of this layer are available. The quantity of mucus within the airways is difficult to assess at necropsy because it may accumulate, agonally, to form plugs and because ciliary activity continues after death (Thurlbeck, 1976). The total amount of mucus in the normal lung probably does not exceed 1.0 ml (Thurlbeck, 1976); patients with chronic bronchitis may have as much as 3.5 ml, but most of this exists as plugs or is trapped in small bronchioles (Matsuba and Thurlbeck, 1973). If we accept these figures, the thickness of the mucus lining of the airways must be very thin indeed.

4. The Pulmonary Macrophage

The origin of macrophages has been studied extensively in recent years. A population of pluri-potential cells in the bone marrow gives rise to a series of progenitor cells from which all blood cells, including monocytes, are derived (Till and McCullough, 1980). Monocytes leave the circulation and enter the tissues where they differentiate into macrophages. The view that macrophages are derived exclusively from the bone marrow has been strongly espoused by van Furth (1970), although a local origin has also been advocated (Volkman, 1976). In the lung, the supply of blood-borne macrophages is augmented by a population of macrophages within the pulmonary interstitial tissue. The adaptive increase in the number of alveolar macrophages that follows inhalation of heavy loads of dust, involves increased delivery of blood monocytes, proliferation of interstitial cells and the initiation of mitotic activity in the free alveolar macrophages (Bowden, 1987).

5. Objectives of the Study

1. To measure the thickness of human bronchial epithelium.
2. To determine the depth and distribution of the basal cells in the bronchial epithelium.
3. To compare these parameters in groups of non-smoking and smoking persons.

The technical problems involved in the development of a method to measure the thickness of bronchial mucus necessitated elimination of this parameter from the study.

B. MATERIALS & METHODS

1. Materials

Surgical specimens were obtained from lobectomy and pneumonectomy cases as follows:

Histopathology Diagnosis	Number	Male	Female
Squamous carcinoma	15	12	3
Adenocarcinoma	6	3	3
Poorly differentiated large cell carcinoma	2	-	2
Metastatic carcinoma	3	1	2
Bronchiolo-alveolar carcinoma	1	-	1
Non-specific inflammatory change	1	1	-
Hamartoma	1	1	-
Total:	**29**		

One additional case (#10) was excluded because the lung was not sliced to give transverse sections of bronchi. One case (#26) was used for electron microscopy. This case was not used for measuring the epithelium.

Smoking and occupational histories were obtained for each case. The data sheet is shown in Figure 2 and the summary of the findings is given in Table 1.

MEASUREMENT OF THE THICKNESS OF THE BRONCHIAL EPITHELIUM
AECB PROJECT # 3H110347246Y

Name **Sex**

Hospital Number **DOB/Age**

Surgical Number

Smoking History:

1. Number of pack/years (approx.)
2. Number of months or years ceased smoking, prior to surgery (if applicable).

Occupational History: Please list major occupations in decreasing order of time commitment, e.g.

Occupation	Approximate dates
1.	19__ to 19__
2.	19__ to 19__
3.	19__ to 19__
4.	19__ to 19__
5.	19__ to 19__
6.	19__ to 19__
7.	19__ to 19__

FIGURE 2

Definitions: Non-Smoker: Never Smoked
Pack-Year: 1 pack/day for 1 year
e.g. 1 pack/day for 5 years = 5 pack years
2 packs/day for 5 years = 10 pack years

2. Methods

2.1 Light Microscopy

2.1.1 Fixation

Following surgical excision, each specimen was delivered to the surgical pathology laboratory where its gross appearances were described and a specimen of the margin taken for routine pathologic diagnosis. The bronchus or bronchi of the specimen were examined to ensure that they were not obstructed; e.g. by blood clot, mucus or tumour. The sizes of cannulae required were determined, each bronchus was cannulated, attached to a rubber tube and connected to the perfusion apparatus. This method was selected for its simplicity in maintaining a constant pressure (Weiss and Tweedale, 1965). Each specimen was fixed by airway perfusion using 10% phosphate buffered formalin (pH 7.0) delivered at a constant pressure of 25 cm water. The apparatus was preassembled as indicated (Figure 3) and tested to ensure that a constant pressure of 25 cm was delivered.

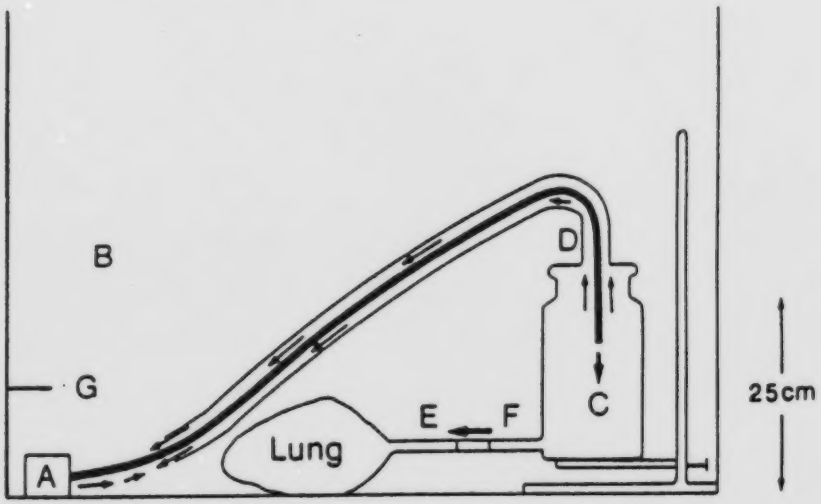


FIGURE 3: PERFUSION APPARATUS

Fixative sufficient to cover the pump (A) was piped into the container (B). The pump was switched on to fill the reservoir (C) which overflowed via tube (D) when the reservoir was full, thus maintaining a constant pressure. The cannulated lung was placed in container B. The position of the reservoir containing

the fixative (F) was adjusted so that the upper level of the fixative was 25 cm above the lower surface of the lung. The single tube (E) from the cannula was attached to the outlet (F) from the reservoir. The fixative inflated the lung and care was taken to ensure that the inflation pressure remained at 25 cm. Fixative was added to the container (B) so that the lung was covered with fixative (G). A cloth towel (wetted with fixative) was used to cover any part of the lung which protruded above the surface of the fixative. The early stages of each inflation by fixative were observed; slight variations in the initial rate of inflation of each specimen was related to variations in bronchial anatomy. Each specimen was perfused for 36 hours, the perfusion apparatus being checked periodically to ensure proper functioning.

2.1.2 Selection of Bronchi

After fixation, the specimen was washed to dilute the formalin and an electric rotary meat slicer was used to cut 0.5 cm slices sequentially, the specimen being oriented to ensure that the maximum number of airways was cut in cross-section. Each slice was labelled in sequence, the gross appearances examined and described. With the occasional exception of a large tumour, which was not fixed adequately because of an absence of patent airways, there was no discernible variation in the quality of fixation. Tissue blocks were selected for routine surgical pathology requirements, following which blocks no larger than 12mm x 10mm x 0.2cm containing airways in cross-section and with a patent lumen were selected from the 0.5 cm slices. The blocks were labelled and placed in fresh 10% neutral buffered formalin until processed for embedding in glycol methacrylate.

2.1.3 Methacrylate Procedure

Blocks of formalin-perfused lung were transferred without washing to a glycomethacrylate infiltration medium for 2 changes of 2 hours each, followed by a third infiltration of 16 hours. The amount of infiltrating medium used was just sufficient to cover the tissue. After 16 hours, the specimens were placed in a vacuum until all air bubbles were removed. The tissue was then placed in embedding medium, on ice, on a shaker for 30 minutes, after which it was transferred to embedding medium in a mold and allowed to polymerize at room temperature in a humidity-free environment. The specimens were left in the molds until immediately before the sections were to be cut.

2.1.4 Microtomy and Staining

Blocks were oriented in the microtome so that bronchi were cut in transverse section. Sections of maximum size obtainable with 12mm glass knives (i.e. 12mm x 10mm) were cut at 1.5 microns thickness, mounted on clean slides and dried on a hotplate at

60-70°C for 15 minutes before staining with toluidine blue-basic fuchsin.

2.2 Electron Microscopy

2.2.1 Fixation

The lung was perfused with 4% phosphate buffered glutaraldehyde pH 6.8, using the same procedure described for formalin.

2.2.2 Selection of Bronchi

Blocks no larger than 2mm x 2mm x 0.5mm were selected from sequential slices, cut on a rotary meat slicer.

2.2.3 Epon-Araldite Procedure

Tissue blocks were washed in buffer, dehydrated through graded alcohols to propylene oxide, infiltrated with Epon-Araldite and embedded in flat molds and polymerized.

2.2.4 Ultramicrotomy and Staining

Blocks were trimmed and oriented in the ultra-microtome so that bronchial epithelium was cut in cross-section. The orientation was checked in 0.5 micron sections using either phase contrast optics on unstained specimens or bright field examination of specimens stained with toluidine in borax. Ultra-thin sections were stained with uranyl acetate and lead citrate and examined in a Zeiss EM10CR electron microscope.

2.3 Morphometry

2.3.1 Selection of Bronchi

Stained sections were examined using bright field microscopy. The details of each section showing the location of bronchi were recorded (Figure 4) and each airway was numbered for future identification. Airways were selected for measurement if the orientation of the epithelial cells on the basement membrane indicated clearly that both were cut in transverse section and that preservation was optimal.

2.3.2 Measurement of Bronchi

A micro-projector, consisting of a Leitz microscope fitted with x 2.5, x 20, x 25, and x 40 obectives, a prismatic projection head and a high-intensity light source (XM-150 Kramer Scientific Corp.) was used to project a micrometer scale on to a sheet of paper. The magnified scale was measured and the total magnification for each objective lens calculated. Once the magnificat-

A.E.C.B. BRONCHIAL EPITHELIUM PROJECT

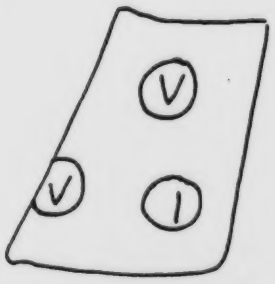
Case # R12

Name Slide # R12-X-29

Diagnosis Squamous Cell Carcinoma

Lung right __ left ✓

Lobe Upper



Each airway measured is coded in the computer

Code

Slide# X-29 lung L lobe U airway# 1 size 2.72mm

FIGURE 4

ion factors were established, the location of the micro-projector was fixed to ensure a constant focal length; the calibration was checked regularly.

A stained section of bronchus was projected on to a sheet of paper. The image of the lumen was then traced in ink and the magnification recorded (Figure 5). Subsequently, the drawing was traced with a cursor on a tablet in a Mop-Videoplan Image Analysis System (Carl Zeiss, Oberkochen) and the diameter of the airway computed from the circumferential measurement by using a standard Kontron Measuring Programme (d-circle).

2.3.3 Mop-Videoplan Image Analysis System

The Mop Videoplan (Kontron Buildanalyse GmBh, Munich, GDR) Image Analysis Computer System is designed for data acquisition and computation of geometric characteristics by tracing structures of images placed on a measuring tablet. The basic units are a control micro-computer, monitor, keyboard and digitizer tablet and printer. The computer has a 64 K byte system memory and 16 K byte memory for representation of graphics and alpha-numerics on a monitor. A 20 M byte hard-disk is divided into four drives, each with a capacity of 5 M bytes; a floppy-disk drive with a capacity of 580 K byte completes the computer system.

Co-ordinate data is generated on the tablet and is continuously calculated into freely selected geometric parameters by the computer. An integrated dual floppy disk drive is utilized for programme and data storage. The standard measurement programmes and measurement data are stored separately on hard disks.

The standard measuring programme Y-1/Y-2 accomodates a parameter display consisting of several different functions with a range of measurements from area type definitions (circumscript of structures), one of which, the D-circle, which computes the diameter of an equivalent circle from a measured area, was used to establish the diameter of airways.

2.3.4 Measurement of Bronchial Epithelium

The measurements taken are indicated in Figure 6. They were grouped according to bronchial diameters and generations (Figure 7), as described by Weibel (1963).

Number of bronchi and cells measured per case are contained in Table 2. Bronchi and cells measured by bronchial generation are indicated in Table 3. The average number of bronchi and cells measured per case and in smokers and non-smokers are indicated in Table 4.

212-X-29

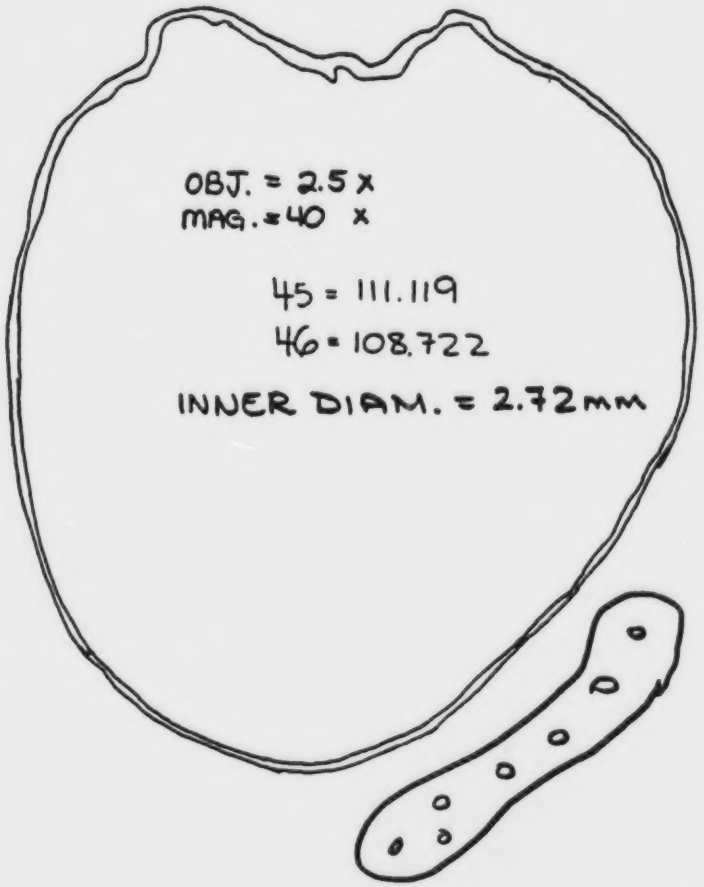
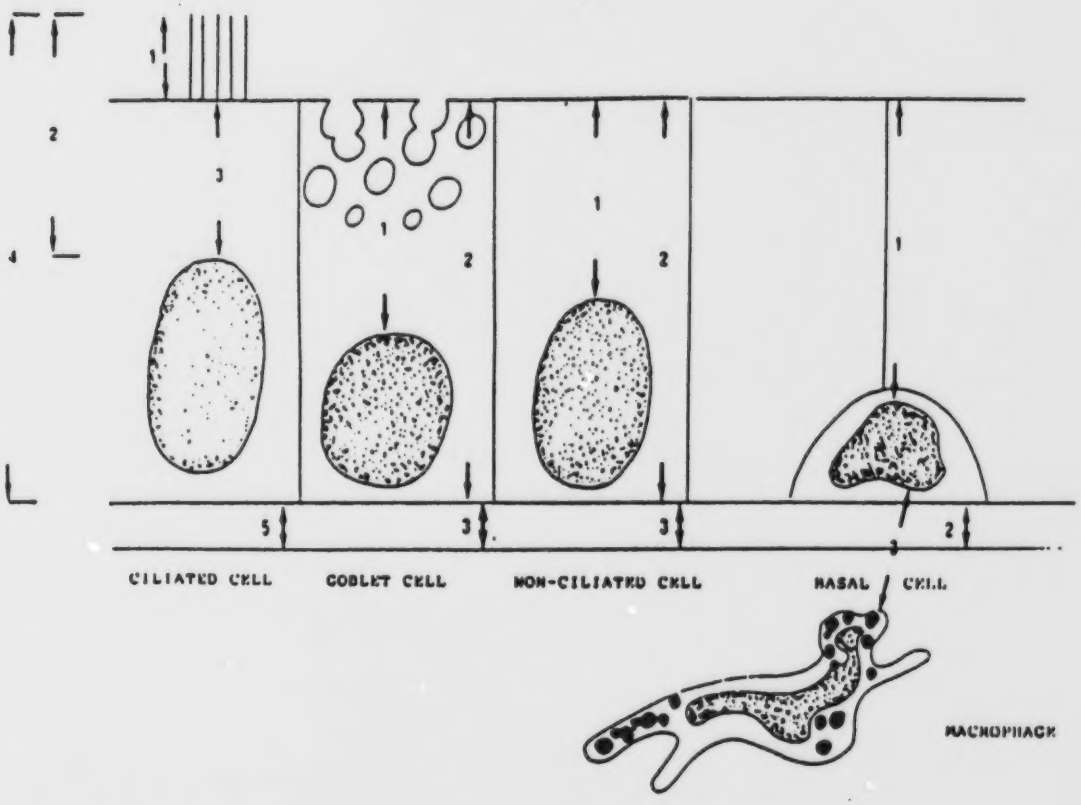


FIGURE 5



Ciliated Cell

1. cilia
2. cilia to nucleus
3. apex to nucleus
4. total cell
5. basement membrane

Goblet Cell

1. apex to nucleus
2. total cell
3. basement membrane

Non-Ciliated Cell

1. apex to nucleus
2. total cell
3. basement membrane

Basal Cell

1. apex of overlying cell to nucleus
2. basement membrane
3. nucleus to macrophage

FIGURE 6 Measurements Taken.

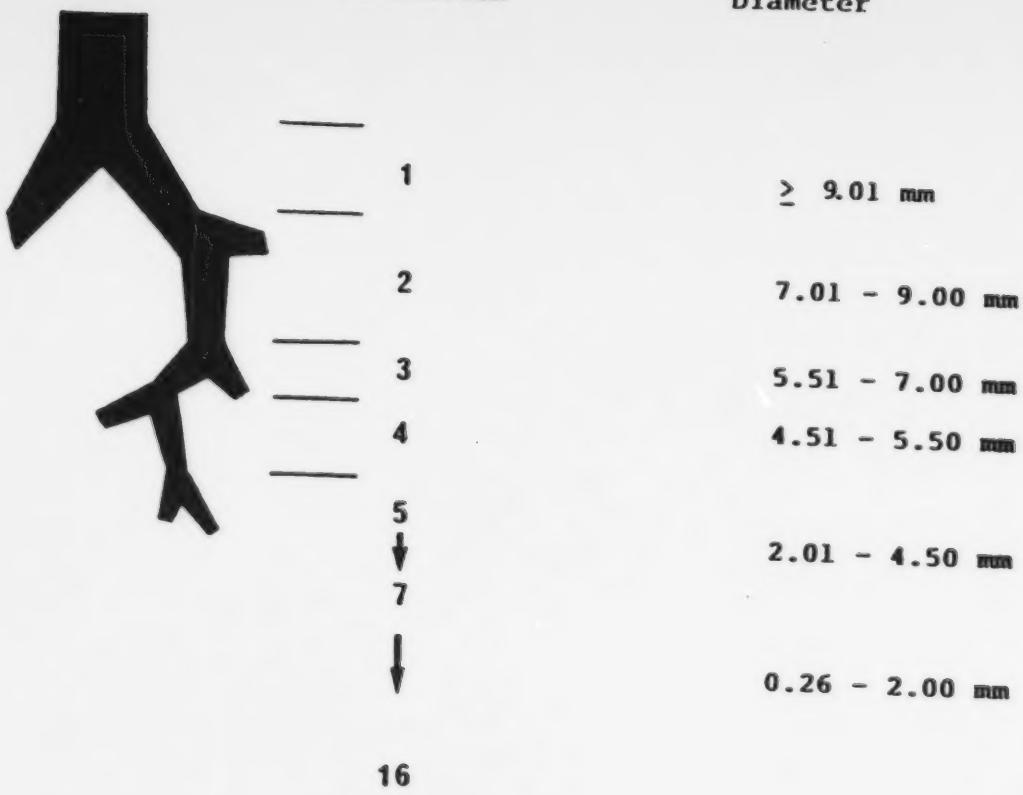


FIGURE 7 Airway Tree: Generation and Diameter.
(after Weibel, 1963)

2.3.5 Cambridge/Olympus Quantimet Image Analysis System

The system used was a Cambridge/Olympus Quantimet Q-10 Image Analyzer (Olympus Corp., Lake Success, N.Y.), interfaced to a Hipad digitizing tablet (Houston Instruments, Austin, Texas). The image measured was produced from an Olympus BH2 binocular microscope using oil immersion optics and an MTI-65 television camera (Olympus Corp., Lake Success, N.Y.). The printer was an Epson EX-800 (Epson Canada Ltd., Willowdale, Ont.). Internally, the system consisted of two CPU's, a Zilog Z-80 and Z-68000, 256-Kilobytes of RAM, and two Quad-density double-sided 5-1/4" floppy disk drives. The operating system was CP/M and the programmes were written in Microsoft Basic (Microsoft Corp., Bellevue, Wash.) and Z-80 assembly language (Digital Research, Calif.).

The image from the MTI-65 television camera was displayed on the RGB monitor of the Q-10 image analyzer, together with a red cross-hair cursor, which represented the current X,Y co-ordinate of the cursor on the Hipad digitizer.

Measurements were made by moving the cross-hairs on the screen cursor to the start and end points of the line length to be calculated, and pressing the cursor button once at each point. The line length was then calculated and stored in a one-dimensional array for subsequent down-loading to a diskfile for statistical analysis. The measurements taken are shown in Figure 6.

2.3.6 Measurement of Thickness of Metaplasia and Hyperplasia

Thickness of bronchial squamous hyperplasia (Figure 11) and metaplasia (Figure 14) apex of cells to basement membrane was determined by measuring the image on the television monitor, using the programme developed for the Quantimet-10 Image Analysis system employed to measure other bronchial epithelium parameters.

2.3.7 Cell Counts

For each bronchus of known epithelial length and computed lumen size, the number of basal cells, ciliated and non-ciliated cells were counted under the microscope.

2.4 Statistical Methods

Case means, by generation and cell type, were computed on the Quantimet Q-10 using standard statistical methods.

One-way analysis of variance and modified t-tests were performed on an Amdahl-4700, using a Bio-Medical Data Package (BMDP Version 5.2), (BMDP Statistical Software, Inc., Los Angeles, Calif.)

2.5 Artefacts of Tissue Preparation

One cm slices of left lower lobe of lungs obtained at autopsy were placed on sheets of paper towelling. Each slide was identified by number and photographed on 35 mm colour slide film, with an accompanying metric scale. Each slice of lung on its sheet of paper towel was carefully placed in a plastic container. Ten percent neutral buffered formalin was added gently until each slice was covered by fixative to a depth of 10 cm, each slice remained submerged. After 36 hours of fixation, each specimen on its paper base was lifted from fixative and photographed with an accompanying scale, as indicated earlier.

Subsequently, multiple samples were taken from each slice with a number 8 skin trephine. Each core sample was numbered and the uppermost surface marked with Mercurochrome and India ink to ensure correct orientation at later stages. Each surface and a metric scale was photographed.

Using standard procedures (see 2.1.3 - 2.1.4) each block was processed through methacrylate, carefully oriented and sectioned at 1.5 µm and stained. Great care was taken to ensure that the section truly represented the face of the tissue block.

2.5.1 Measurement of Lung Slices and Sections

Using a projector (Leitz Pradolux RT 300), colour slides of fresh and fixed lung slices and core samples were projected onto a sheet of paper. The magnified scale was measured and the total magnification calculated and the image of each lung slice and each block was traced in ink and the magnification recorded. Stained microscope sections were projected and traced as described previously (see 2.3.2).

Subsequently, each drawing was traced with a cursor on a table on a Mop-Videoplan Image Analysis System and the area of each slice, block and section computed from circumferential measurement, using a standard Kontron measuring programme as described previously (see 2.3.3). Each measurement was made five times.

The area of each slice, fresh and fixed, and core samples and sections was compared and expressed as a percentage.

C. RESULTS

1. Epithelial Morphology

Accurate measurement of the various parameters of epithelial thickness demands excellent histological preparations. Rapid fixation and the use of methacrylate embedding ensured optimal conditions for the required examinations and measurements (Fig-

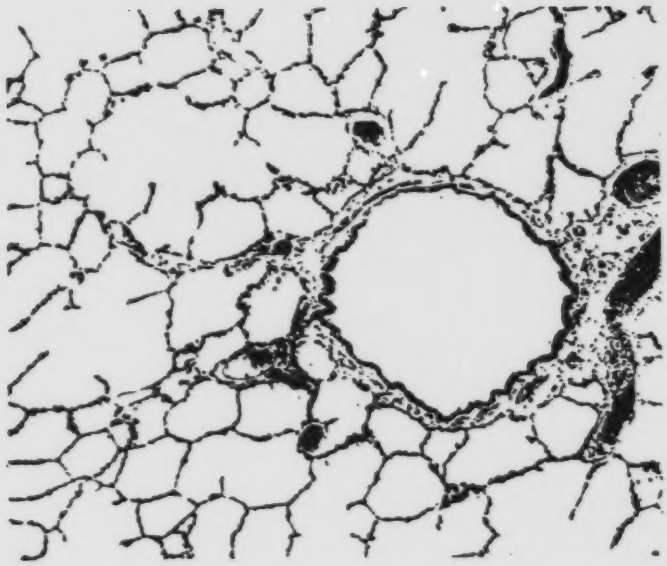
ure 8). The morphology of the bronchial epithelium was remarkably uniform in all of the cases examined (Figures 8, 9, 10) but, as expected, focal areas of epithelial hyperplasia (Figures 11, 12, 13) and metaplasia (Figures 13, 14, 15) were observed.

1.1 Basal Cells

During light microscopic examination of bronchial epithelium, in addition to typical small pyramidal basal cells there were larger elongated cells also occupying a basal location (Figures 16, 17, 18). The elongated cells were distributed circumferentially, and were readily distinguished from the typical pyramidal basal cells (Figure 16). Both types were enumerated.

Ultrastructural examination revealed that the typical pyramidal basal cells conformed to established criteria for basal cells with regard to shape, organelles and the relationship to adjacent cells. (Lentz, 1971; Jeffrey and Reid, 1975; McDowell et al, 1978, Kawanami, 1979; Plopper et al, 1983) The nuclear cytoplasmic ratio was high, the scant cytoplasm was moderately electron dense, and organelles, including mitochondria, were relatively sparse. Rough endoplasmic reticulum and Golgi apparatus were poorly developed; occasional lysosomes were seen, polyribosomes were evident and bundles of tonofilaments were randomly distributed. The cells were attached to the underlying basement membrane by hemidesmosomes, and desmosomes were seen on the lateral surfaces of the cells. Some tonofilament bundles were seen to terminate at these junctions.

Electron microscopy of the elongated basal cells showed that they were located on the epithelial side of the basement membrane of the columnar cells (Figure 19). The nucleus was spindle shaped and the cytoplasm was predominantly polar in distribution and extended into processes. The cytoplasm was moderately dense and the organelles (Figure 20) included some mitochondria, lysosomes were evident and a Golgi apparatus was occasionally seen (Figure 21). Some rough endoplasmic reticulum was evident and polyribosomes were numerous. Cytoplasmic filaments were abundant (Figure 22), bundles of tonofilaments were evident, some of which terminated at desmosomes. In some cells the filaments occupied much of the cytoplasm (Figure 23). Centrioles were sometimes seen. Desmosomes were present where the processes of basal cells came into contact with one another (Figure 24). The cells were attached to the basement membrane by hemidesmosomes (Figure 21). Desmosomes were also present between the elongated basal cells and overlying columnar epithelial cells (Figure 25), as well as between adjacent columnar cells. Desmosomes or other junctions between columnar cells and basement membrane were never seen.



8

FIGURE 8 Bronchus and Alveoli.

Microanatomical preservation typical of preparations used in the study. x 56

9

FIGURE 9 Bronchial Epithelium.

a) ciliated columnar cell
b) goblet cell
c) basal cell x 500

10

FIGURE 10 Bronchial Epithelium and Debris Laden Macrophage in the Interstitium. x 500

11

FIGURE 11 Goblet Cell Hyperplasia. x 500

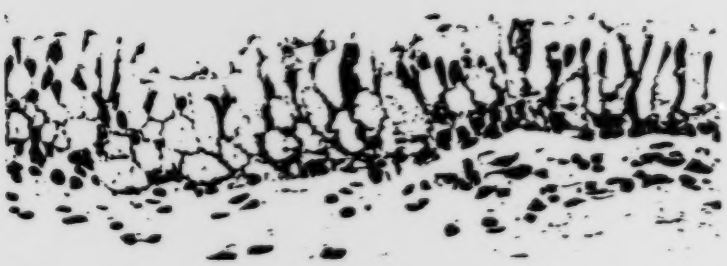


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FIGURE 12 Goblet Cell Hyperplasia. x 312

13

FIGURE 13 Goblet Cell Hyperplasia and Squamous Cell Metaplasia. x 125

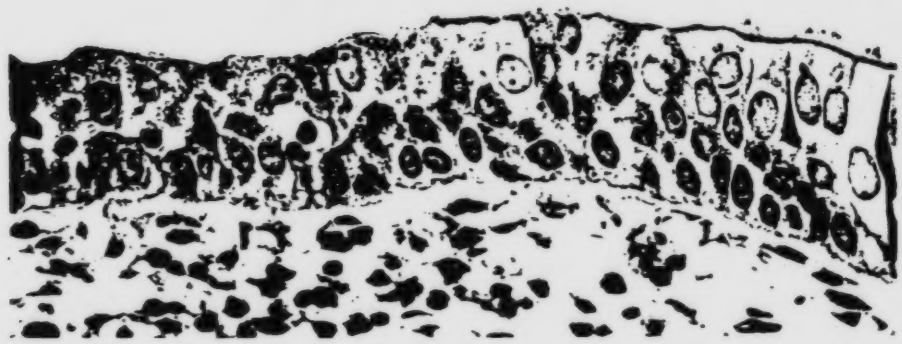

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FIGURE 14 Squamous Cell Metaplasia. x 500

15

FIGURE 15 Squamous Cell Metaplasia. x 312

16

FIGURE 16 Bronchial Epithelium with Typical and Atypical Basal Cells. x 500

17

FIGURE 17 Bronchial Epithelium with Elongated Basal Cells. x 1250

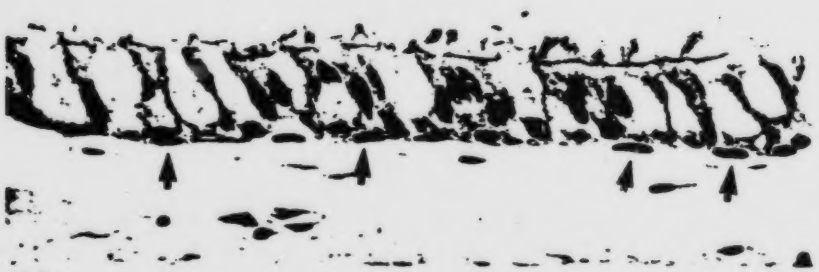

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FIGURE 18 Bronchial Epithelium with Circumferential Distribution of Elongated Basal Cells. x 500

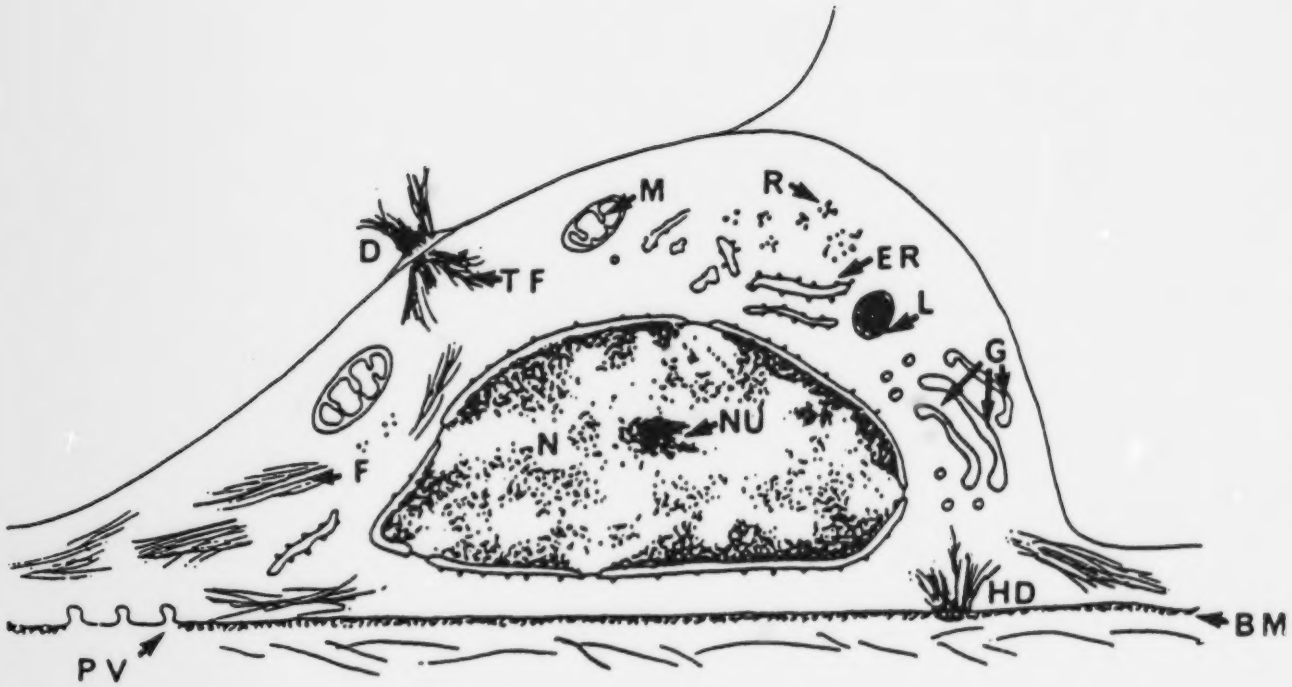
KEY TO FIGURES 19, 21 - 25.

BM	Basement Membrane
C	Cilia
CC	Columnar Cell
D	Desmosome
EBC	Elongated Basal Cell
ER	Rough Endoplasmic Reticulum
F	Filaments
G	Golgi Apparatus
HD	Hemidesmosome
L	Lysosome
M	Mitochondria
N	Nucleus
R	Polyribosome
TF	Tonofilament

19

FIGURE 19 Bronchial Epithelium.

Elongated Basal Cell and its Location in the Epithelium. Bar (-) = µm



BM	Basement Membrane
D	Desmosome
ER	Endoplasmic Reticulum
F	Filaments
G	Golgi Apparatus
HD	Hemidesmosome
L	Lysosome
M	Mitochondrion
N	Nucleus
NU	Nucleolus
PV	Pinocytotic Vesicle
R	Polyribosomes
TF	Tonofilaments

FIGURE 20 Diagram of Cell Organelles and Surface Specializations.

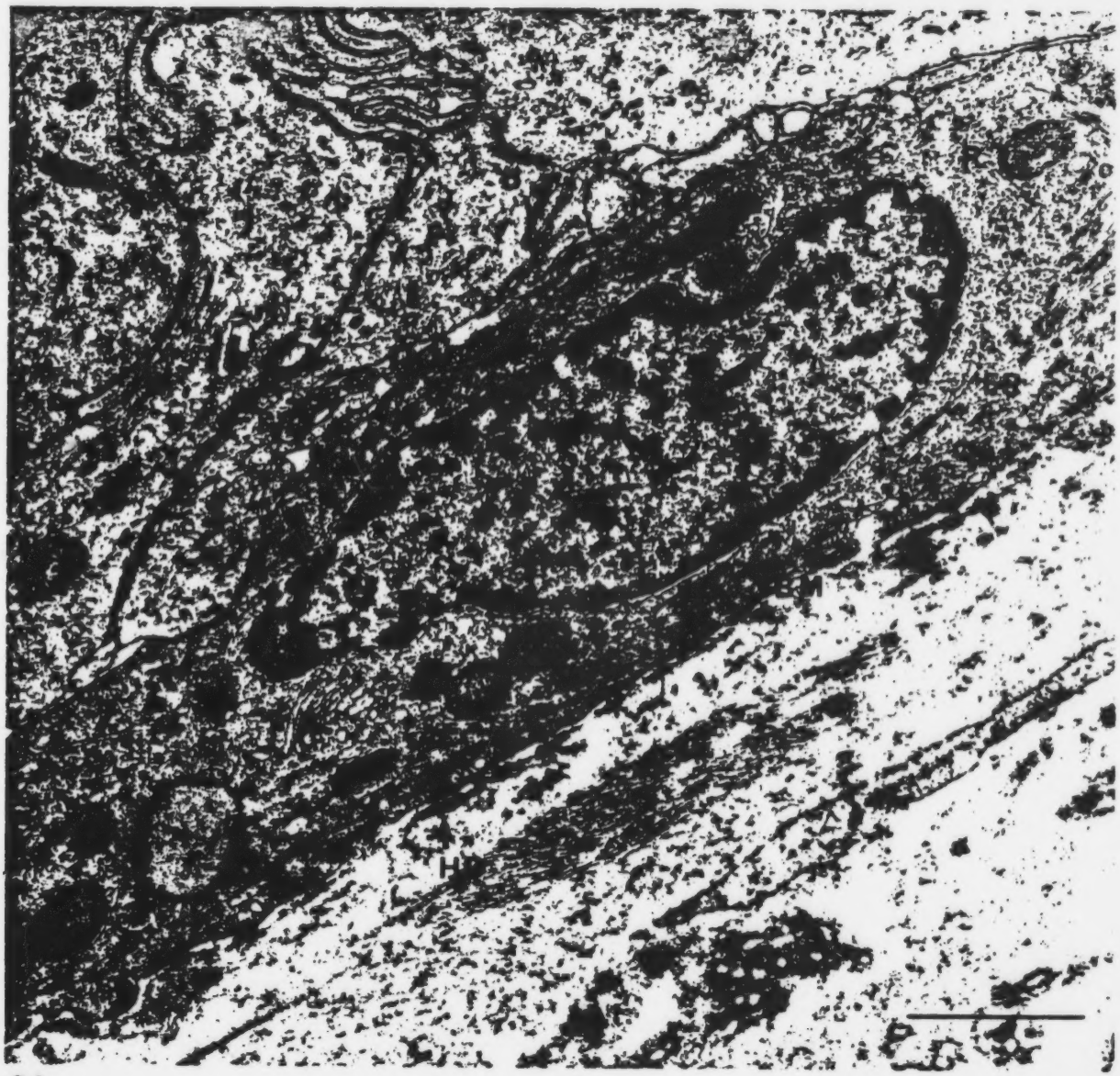


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FIGURE 21 Elongated Basal Cell.

Organelle-rich Polar Cytoplasm and Relationship of the Cell to the Basement Membrane.

Bar (-) = μm

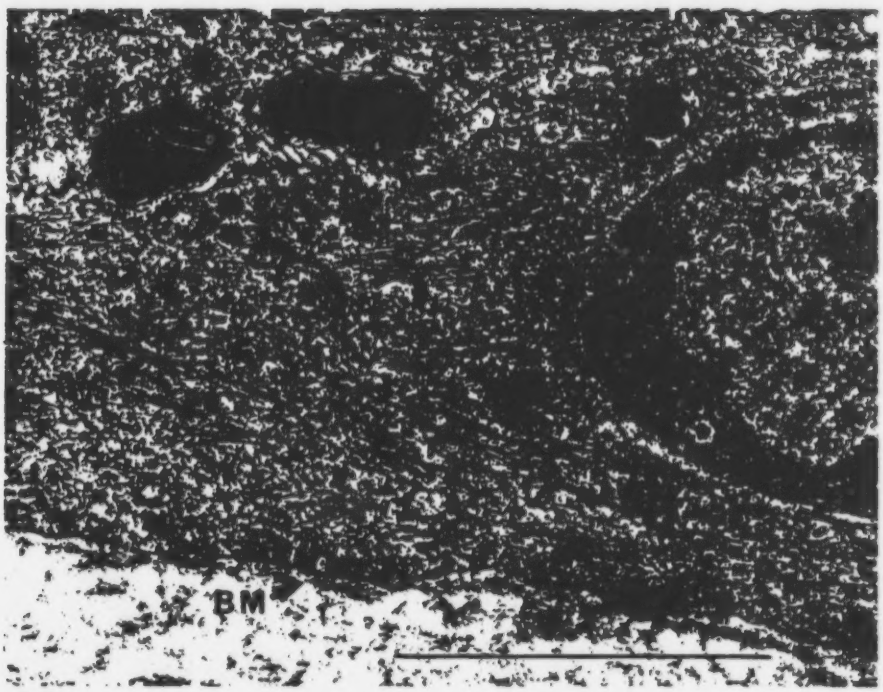

FIGURE 22 Filamentous Cytoplasm in Elongated Basal Cell.

Bar (-) = μm

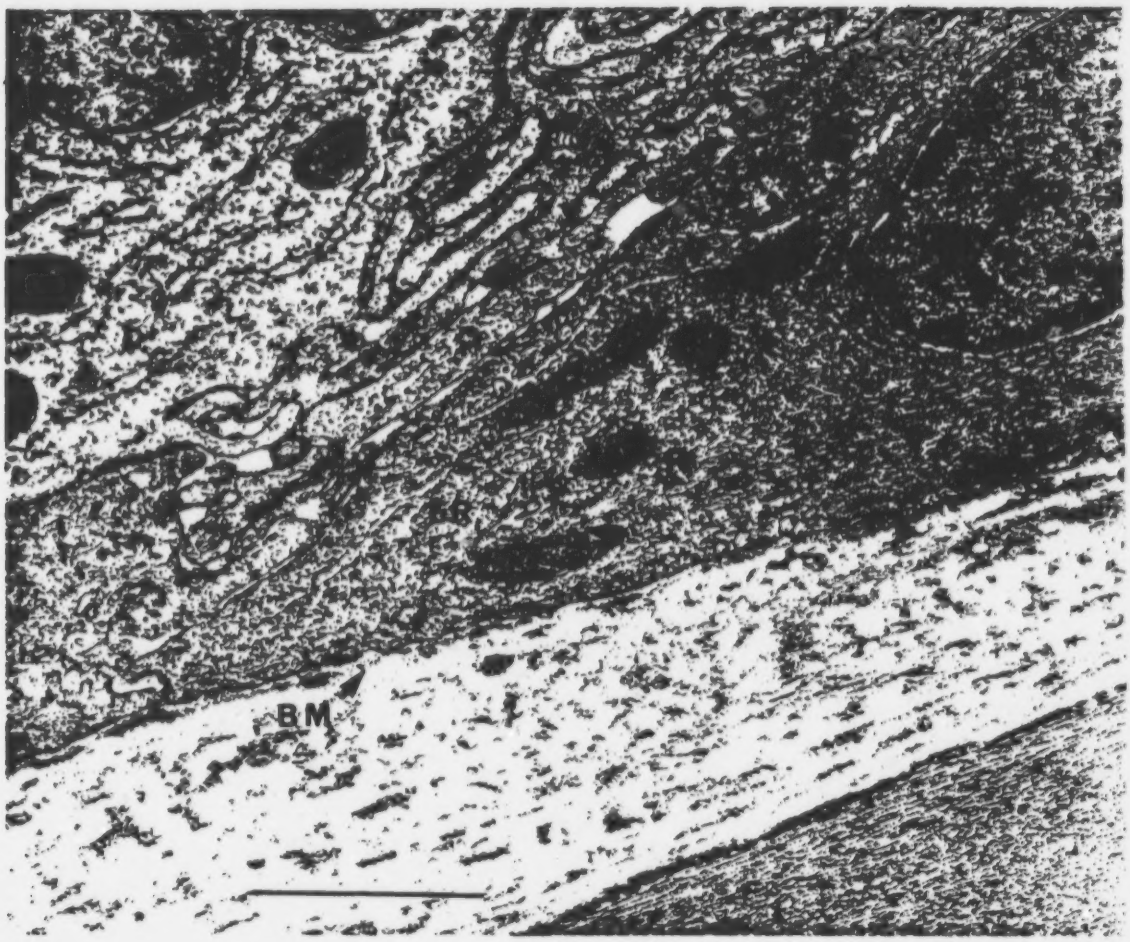

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FIGURE 23 Highly Filamentous Cytoplasm of Elongated Basal Cell.

Bar (-) = µm

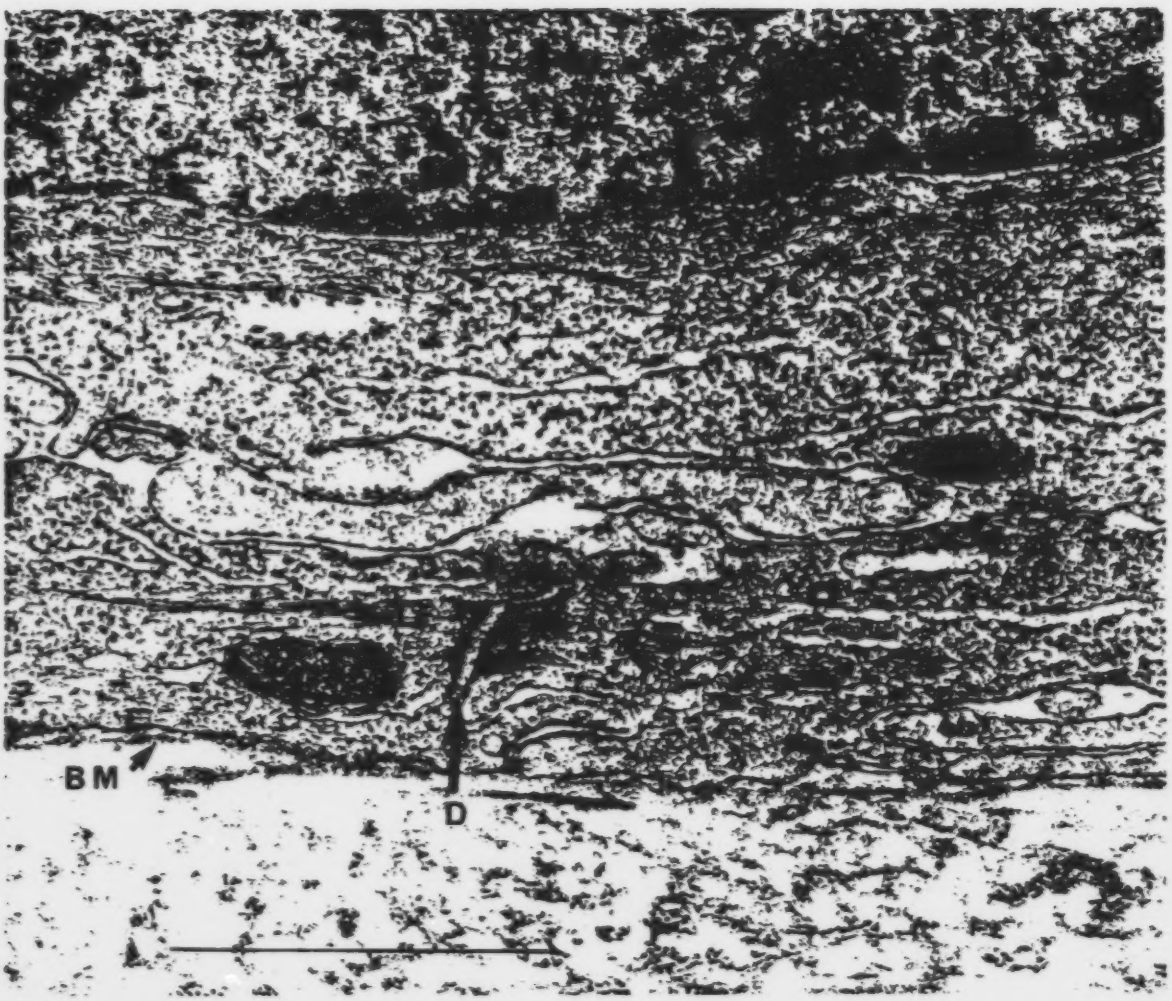


FIGURE 24 Contact Between Basal Cell Processes.

Desmosome and Tonofilaments.

Bar (-) = µm

25

FIGURE 25 Bronchial Epithelium.

Attachment of Elongated Basal Cell to Basement Membrane and Columnar Epithelial Cells.

Desmosomes Between Adjacent Columnar Cells.

Bar (-) = μm

2. Epithelial Measurements

2.1 Artefacts of Tissue Preparation

In our study, the lungs used were surgical specimens for which there were diagnostic requirements -- thus the fixation procedure utilized was selected because of its relative simplicity for fixing lungs under controlled conditions for morphometric purposes while enabling routine Pathology assessments.

The principle objective of fixation is to minimize alterations to structural relationships within the entire tissue sample. The comparable morphology of the large number of lung samples examined microscopically indicates that all parts of the lung were equally well fixed. Taking into account that all specimens received identical processing, sectioning and staining, any artefacts produced should be present in all tissue samples. The data in the morphometric study are all based on point-to-point measurements; thus the measured effects of fixation upon lung tissue and the effects of processing upon fixed specimens enabled the processing artefact to be determined which could be applied to the measurement data as follows:

TISSUE PREPARATION ARTEFACT *

	Shrinkage	Expansion
Fixation (n=20)	-3.85% ± 0.52% S.E.	
Methacrylate Processing and Microtomy (n=60)		+3.1% ± 1.77% S.E.

* (preparation artefact 3.85% - 3.1% = 0.75%)

2.2 Smokers VS Non-Smokers

Measurements of epithelial cells were made in 25 smokers and 3 non-smokers and the figures submitted to an analysis of variance as described earlier. The figures for all cell types, analyzed by pack-years and bronchial generation are shown in Tables 5A to 5F. Since no significant difference was found between the groups analyzed, the 28 cases were considered to represent a homogeneous group and the figures depicted in subsequent tables and figures are derived from measurements made on smokers and non-smokers; a total of 28 cases.

2.3 Analysis by Cell Type and Bronchial Generation

In Table 6, the measurements for each type of epithelial cell are charted according to the numbers of bronchial generation from

generation 1 to 16 (Weibel, 1963). These generations correspond to bronchial diameters of 9.01 mm to 0.26 - 2.00 mm.

The analysis of variance between individual bronchial generations are given in Table 6.1 and statistically significant differences of epithelial measurements for all bronchial generations are shown in Tables 6.2A to 6.2D.

With progressive divisions of the bronchi, the epithelium decreases in thickness; for ciliated cells, non-ciliated cells and basal cells the differences between the measurements in generation 1 and generations 7-16 are significant ($p = <0.05$) The total thickness of ciliated cells and their basement membranes (distances 4 and 5 in Figure 6) in generation 1 is 41.89 $\pm$ 2.53μm and in generations 7-16 the value has decreased to 29.23 $\pm$ 1.50μm ($p = 0.006$). For typical basal cells, the respective values are: 44.57 $\pm$ 2.88μm and 31.39 $\pm$ 1.26μm ($p = 0.006$).

A more detailed analysis, including the critical measurement of apex to nucleus is provided in Table 7A to 7E , Tables 7.1A to 7.1D, and Tables 7.2A to 7.2D.

2.4 Ciliated Cells

Cilia tend to be longer in generation 1(6.52μm) than in generations 7-16(5.58μm, ($p = 0.004$). The mean distance from the cellular apex to the nucleus in (distance 3 in Figure 6) in generation 1 is 12.86μm, almost twice the measurement recorded for generations 7-16 (6.53μm)
($p = 0.001$).

2.5 Non-Ciliated Cells

The distance between the apex of the cell and the nucleus (distance 1 in Figure 6, Non-Ciliated Cell) does not vary significantly throughout the bronchial tree (10.08μm); measurements from the apex to the basement membrane are smaller in generations 7 - 16 ($p = 0.0038$).

2.6 Goblet Cells

The distance between the apex of the cell surface and the nucleus (distance 1 in Figure 6, Goblet Cell) does not vary significantly from generation 1 (19.83μm) through generations 7-16 (18.10μm).

2.7 Basal Cells

In view of the presumed role of the basal cell as the ultimate progenitor of all bronchial epithelial cells, special attention was given to the enumeration of the cells by bronchial generation and in relation to smoking history. The figures are shown in

Tables 8A and 8B. No significant trends are detected in the analysis of these figures, the percentages of typical/atypical basal cells bear no clear relationship to smoking history or to bronchial generation.

2.7.1 Typical Basal Cells

In generation 1, the nucleus of the basal cell is 26.65μm from the surface (distance 1 in Figure 6, Basal Cell), whereas in generations 7-16, the distance is only 17.46μm ($p = 0.0019$) (Table 7.2C). There are significant differences between generation 2 and generations 7 - 16, generation 3 and generations 7 - 16, and generation 5 and generations 7 - 16 (Table 7.2C).

2.7.2 Atypical Basal Cells

The distances between the nuclei of these cells and the surface of the overyling cells are significantly different between generations 1 and 3, generation 1 and generations 5 - 7, and generation 1 and 7 - 16, and generation 4 and generations 7 - 16 (Table 7.2D).

2.8 Thickness of Epithelium and Anatomical Location

Comparisons between the thickness of epithelium in each generation of bronchi from different lobes of the lung (Tables 9A to 9F) revealed that there were no significant differences; neither were there significant differences between the thickness of basement membranes.

2.9 Basal Cells and Dust-Laden Macrophages

Very few collections of dust-laden macrophages were identified in these lungs. The measurements that were made were limited to generations 4 - 6 (Table 10). The distances from these macrophages to typical basal cells (distance 3, Figure 6, Macrophage) fall within the range 4.11 - 9.03μm; for atypical basal cells, the range was 3.4 - 4μm. There are no significant differences between the measurements made in different bronchial generations.

2.10 Metaplasia and Hyperplasia

The measurements obtained of the thickness of metaplastic and hyperplastic epithelium (Figures 11 and 14) are fewer in number than those given for the normal cell types. This is inevitable, because of the patchy nature of the changes and, in many of the cases, metaplasia and hyperplasia were unusual. Nevertheless, we have included the figures because they may provide useful comparisons with the measurements of the normal epithelium at various levels of the bronchial tree. Table 11 shows the measurements for smokers only because metaplastic changes in the non-smokers

were too few for analysis. There is no significant variation between the measurements recorded for generations 1 through 16. The distance between the inner surface of metaplastic epithelium and epithelial basement membrane varies between 27.5 μm and 49.8 μm (Figure 26); the hyperplastic epithelium shows a range of 41.97 μm to 50.2 μm (Figure 26).

D. DISCUSSION

The study is based upon measurements made on fresh pulmonary tissue removed by pneumonectomy or lobectomy from 28 patients. Not suprisingly, the most common diagnosis was primary carcinoma of the lung (24). There were three cases of metastatic cancer, one hamartoma and one lung showed non-specific inflammatory changes only. Of the 28 patients, three were non-smokers. The various parameters of epithelial thickness, including measurements from the surfaces of the cells to their nuclei, were measured and the figures for the non-smokers were compared with those obtained in smokers, grouped by pack-years. No significant differences were found between these groups and the non-smokers indicating that, for subsequent analyses, the 28 cases could be treated as a homogeneous sample.

In an earlier study, Gastineau et al (1972) measured the thickness of bronchial epithelium, the length of cilia and the thickness of the basal cell layer in different regions of the bronchial tree. These measurements were made on paraffin sections and the artefacts induced by preparation were assessed by measuring the diameter of erythrocytes in the sections as compared with the "physiological" diameter of red cells. However, the actual amount of shrinkage was not reported, and the precise method of preparation of the tissue sections was not described. Bronchial cilia were 7.5 μm and the median thickness of basal cells was 7.5 μm for all except the main stem bronchus, where it was 10 μm. Basal cells within range of alpha radiation from a source at the surface of the mucus layer were determined by subtracting the thickness of the basal cell layer (7.5 μm) from the sum of the estimated average mucus layer thickness (7 μm), the height of cilia (7.5 μm) and the epithelial thickness. A 6.0 MeV-218 Po alpha particle has a range of 47 μm and a 7.69 MeV-214 Po alpha particle has a range of 70.8 μm (Lea, 1955). Thus, basal cells in a 40 μm thick epithelium were considered to be outside the 47 μm range of a 6.0 MeV-218 Po alpha particle and, basal cells of a 63.8 μm thick epithelium outside the 70.8 μm range of a 7.69 MeV-214 Po alpha particle.

Over two-thirds of the basal cells in the lobar and segmental bronchi, i.e., generations 2 - 16 (Weibel, 1963) appeared to be within range of the 7.69 MeV-214 Po alpha particle, and about one-fifth were within range of the 6.0 MeV-218 Po alpha particle. The proportions would have been higher if the radioactive

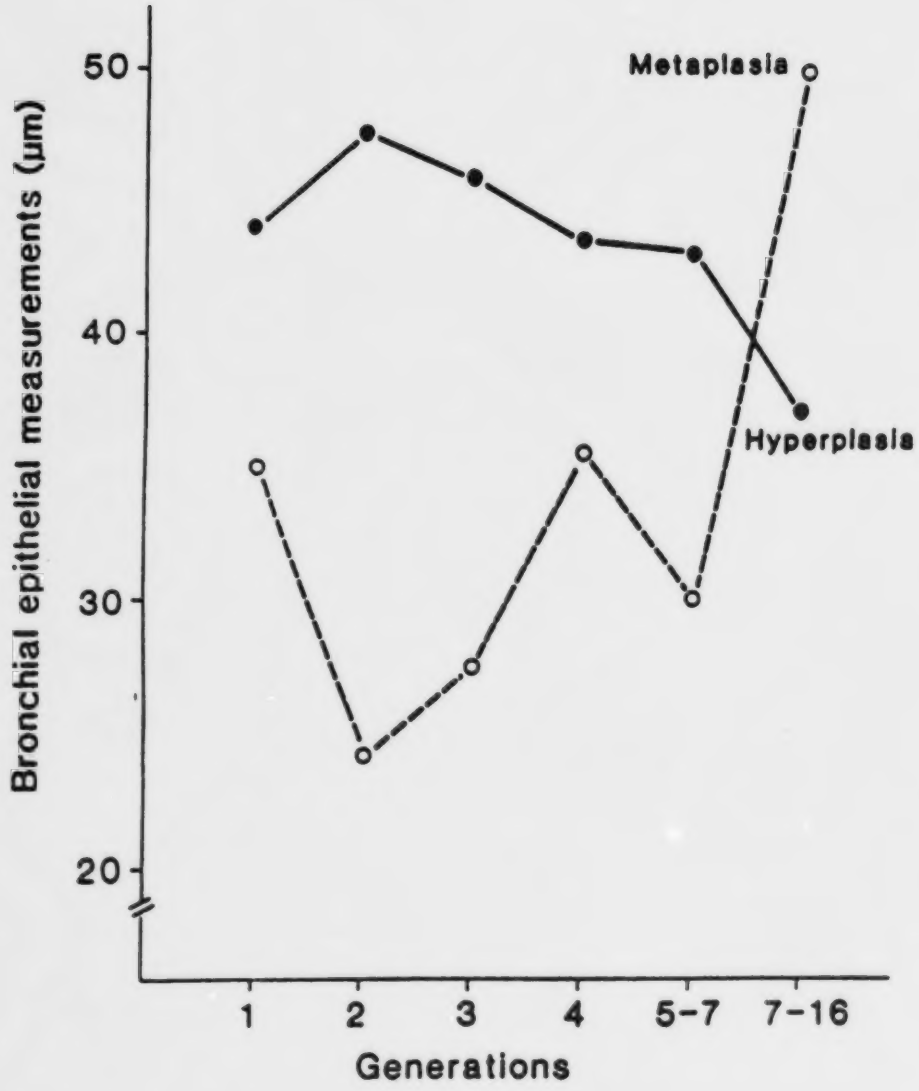


FIGURE 26 Bronchial epithelial thickness
Metaplasia and hyperplasia
Smokers

substances were soluble in the mucus and, therefore, not at the surface. Basal cells in the thicker epithelium of main stem bronchi fell outside the range of alpha particle irradiation, but some areas of metaplasia were sufficiently thin to permit exposure of basal cells.

The thickness of the epitheliium tended to decrease as the branches got smaller; and there were no significant differences between the thickness of epithelia in lobar, segmental and sub-segmental bronchi.

In the current study, establishing an accurate correction factor for artefacts of preparation was necessary so that measurements would be comparable to the in vivo state. Measurements made of fixed and unfixed lung samples revealed that fixation in 10% neutral buffered formalin caused 3.85% ($\pm$ 0.52 S.E.) shrinkage. However, measurements made of fixed lung samples and stained methacrylate sections from the same blocks revealed that there was an increase in size of 3.10% ($\pm$ 1.77 S.E.). Thus, the correction factor applied to all measurements involved a shrinkage of only 0.75%, a very small amount considering the overall distances of susceptible nuclei from possible radiation sources. We cannot compare this shrinkage factor with that used by Gastineau, et al (1972), because they provided no data; it is generally understood however, that shrinkage caused in the preparation of paraffin sections is substantial because of the effects of solvents and of heat. Variations in the time tissue is subjected to various parts of the process also influences the production of artefacts. Furthermore, the relatively poor resolution obtained by light microscopy of routine paraffin sections restricts the accuracy of measurements.

The selection of the methacrylate procedure and its implementation under carefully controlled conditions reduced artefacts to a minimum, and contributed to the accurancy of measurements because of the improved resolution obtained with 1.5 μm sections. For practical purposes, therefore, the measurements made are accurate within the confines imposed by the resolution of the light microscope.

Consistent technical preparation is imperative, e.g. it was found that differences in embedding procedure, section thickness and section manipulation affected the magnitude of artefacts. In this study, each specimen received identical preparation and all sections were cut at the same thickness and manipulated in exactly the same way. It should be emphasized that the correction factors established apply only to the precise method described; they are not transferable to specimens of lung prepared in a different manner, and they cannot be applied to other tissues.

Our results agree with those of Gastineau et al, (1972), in that the thickness of bronchial epithelium decreases progressively with successive generations. However, where direct comparisons are possible, measurements made by the present authors indicate that the bronchial epithelium is much thinner than shown previously by Gastineau et al (1972).

COMPARISONS OF EPITHELIAL THICKNESS

Bronchus	Mean Thickness (μm)		% Difference
	Gastineau et al (1922)	Bowden & Baldwin	
Main Stem (Generation 1*)	80	40	-50
Lobar (Generation 2*)	50	39	-22
Segmental (Generation 3*)	50	36	-28
Subsegmental (Generations 4-16*)	50	33**	-34

* Weibel 1963
** Average of means for generations 4(34), 5-7(37), 7-16(29)

Of more importance, however, are the data relating to the distance from the cell surface to the underlying nuclei. The study of Gastineau, et al (1972) concentrated on the distance of basal cells from the mucociliary surface and its relation to radiation effects. No precise measurements were made of the distance of basal cell nuclei or of other epithelial nuclei from the cell surface.

Studies in experimental animals indicate that, in addition to basal cells, there are other proliferating populations, including non-ciliated columnar cells with and without secretory granules (Bindreiter, et al, 1968; Lane and Gordon, 1974; Becci and McDowell, 1978; Boren and Paradise, 1978; Donnelly et al, 1982; Evans et al, 1986). It has been shown that secretory cells are the primary progenitors of other epithelial cells in the conducting airways (Keenan, et al, 1982a, 1982b; and McDowell et al, 1984, 1985).

In the present study, measurements included the distance from surface to nucleus for all epithelial cells types. When measurements from smokers and non-smokers were combined, with the exception of goblet cells, the measurements for generations 7 - 16 were significantly smaller than those for generation 1. The downward trend through descending bronchial generations is shown in Figure 27. The greatest distance from the surfaces of cells to their nuclei are in bronchial generation 1; for basal cells the distance is less than 27μm; for goblet cells it is less than 20μm, and the nuclei of ciliated and non-ciliated cells are not more than 13μm from the surface. In generations 7 - 16, the distance of all cells is between 7 - 18μm.

When smokers are considered separately (Figure 28), with the exception of goblet cells, the downward trend in the distance from cell surface to nucleus for all cells in descending generations is observed. The greatest distances are in generation 1. For basal cells, the distance is less than 35μm. For goblet cells, it is less than 22μm, and the nuclei of ciliated and non-cilated cells are not more than 20μm from the surface. In generations 7 - 16, the distance of all nuclei from the luminal surface is between 6 - 24μm.

In non-smokers (Figure 29), the greatest distance from cell surface to nucleus may be different. It is greatest in generation 2 for all cell types, although it is likely the lesser distances recorded for generation 1 were influenced by the small size of the sample measured and may, therefore, not be truly representative. For basal cells, the distance is less than 35μm, Goblet cell and ciliated cell nuclei are less than 20μm, whilst non-cilated cell nuclei are less than 12μm from the cell surface. The downward trend in distance is observed through generations 7 - 16, when the nuclei of all cells are between 6 - 22μm from the surface.

The average size of the nucleus in different epithelial cell types is summarized in Figure 30. The nucleus of each cell type has a different size, and there are no significant differences between nuclear sizes in different generations for all cell types, with the exception of non-ciliated cells. The following chart summarizes the data in a form that may be useful in determining their relevance to the range of radioactive particles impinging upon the surface of the bronchial epithelium. Since the data derived are from measurements made from the cell surface to the underlying nuclei, an average figure for the height of the cilia has been used (6μm) and the thickness of the mucus layer has been estimated as 5μm. The small shrinkage factor (0.75%) has been ignored.

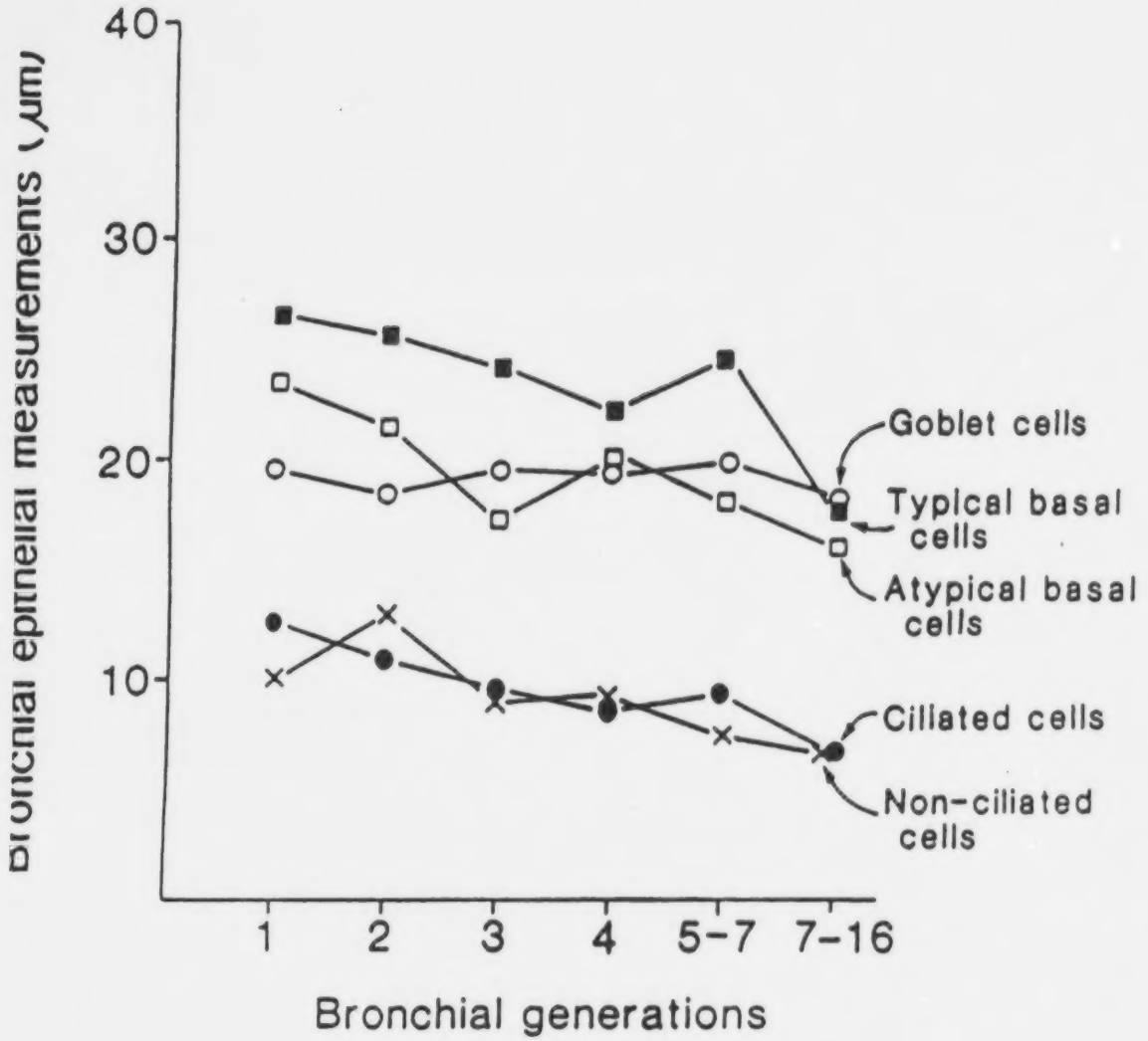


FIGURE 27 Measurements: cell surface to nucleus. Smokers and non-smokers combined.

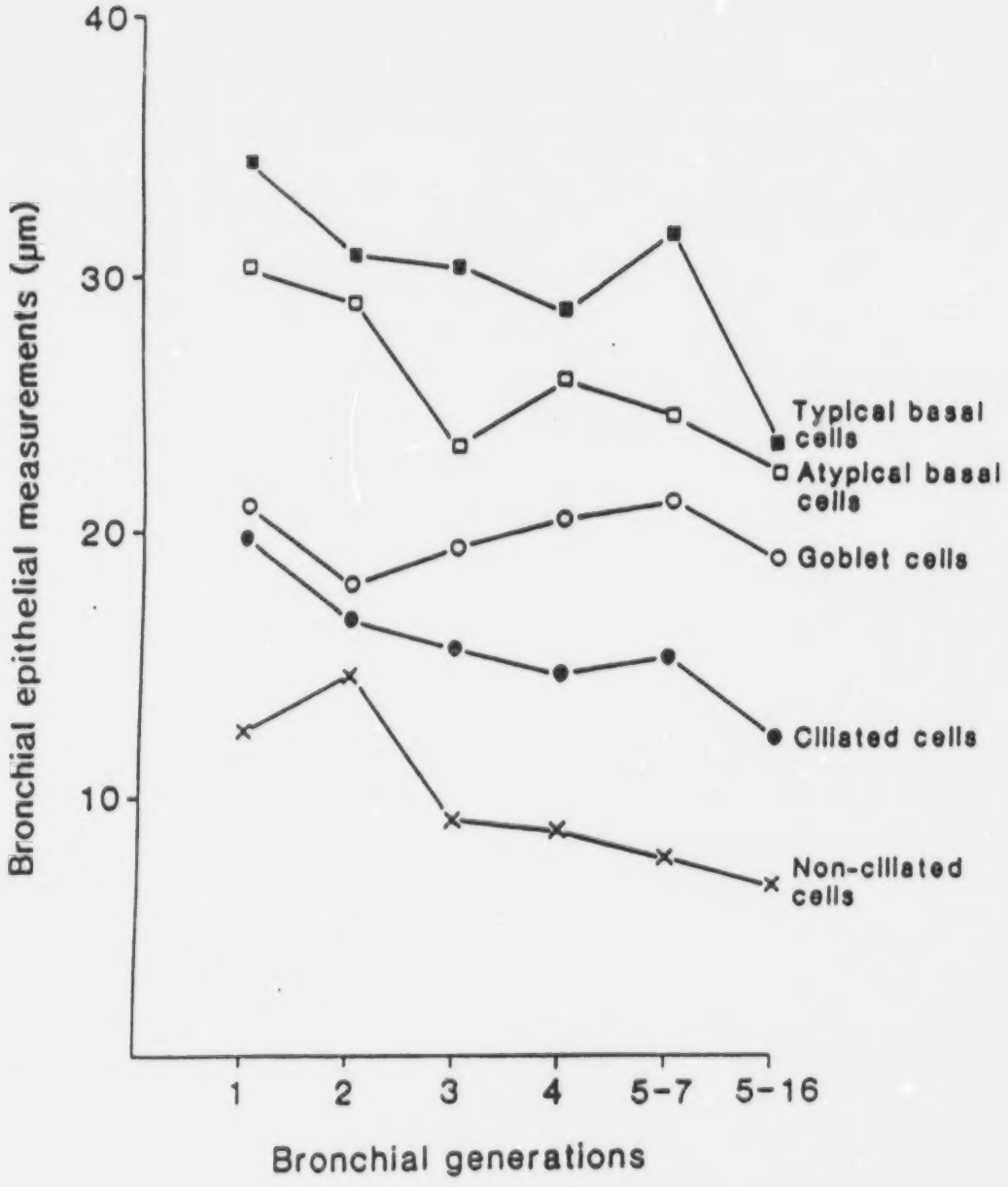
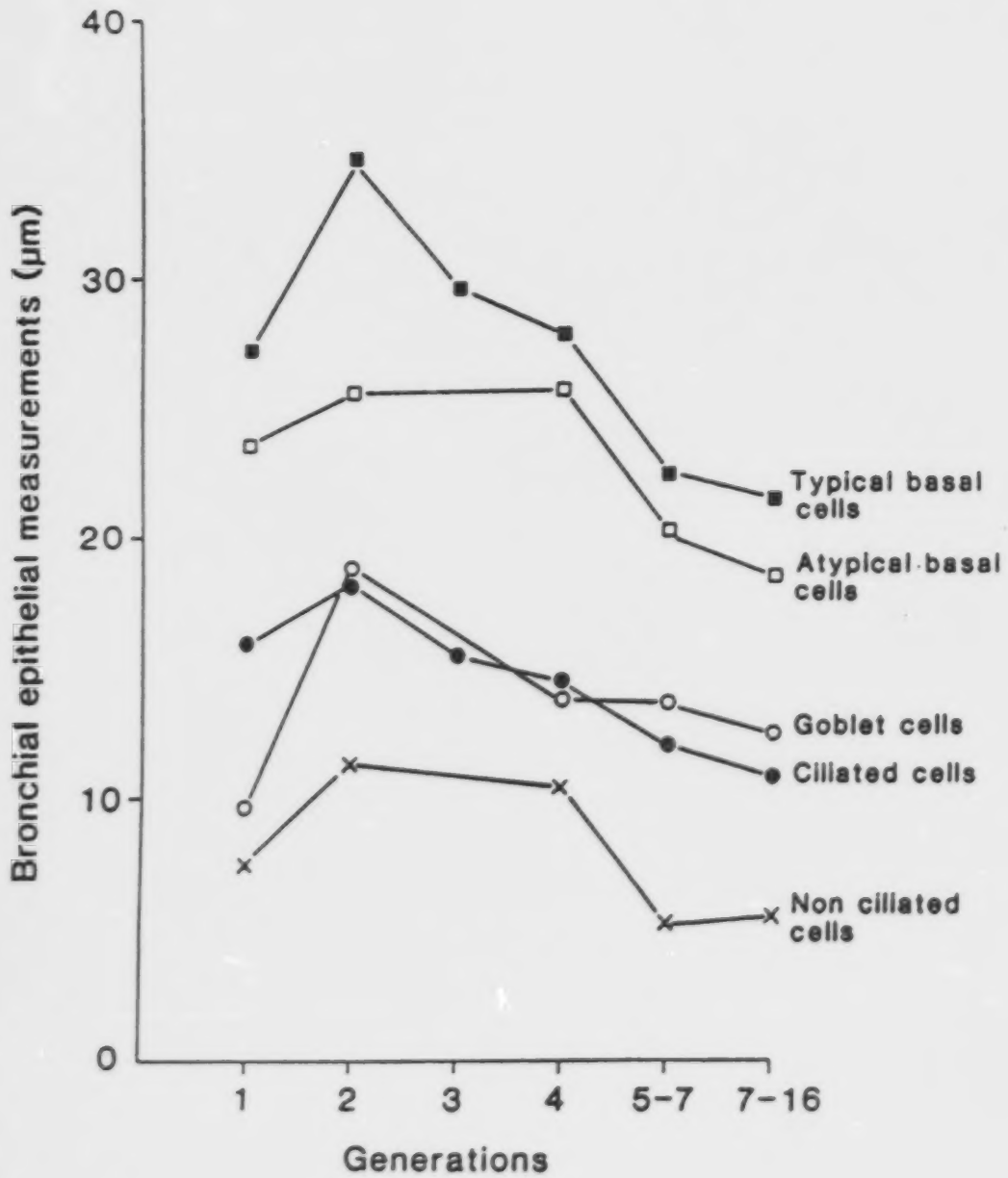


FIGURE 28 Measurements: cell surface to nucleus. Smokers.



PIGURE 29 Measurements: cell surface to nucleus. Non-smokers.

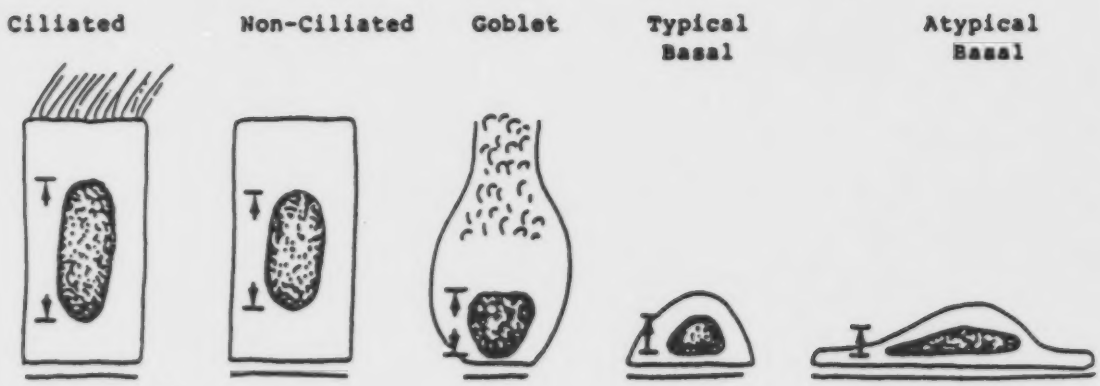


FIGURE 30 Bronchial Epithelial Cells.

nuclear size (μm)

mean ± Standard Error

AVERAGE MEASUREMENTS

Cell Type	Distance of Nucleus from Mucociliary Surface (μm) (n=cells measured)	
	Generation 1	Generations 7 - 16
Basal Cells (typical)	38(n=289)	28(n=470)
Basal Cells (atypical)	34(n=80)	27(n=305)
Goblet Cells	31(n=197)	29(n=260)
Ciliated Cells	24(n=393)	18(n=1246)
Non-Ciliated Cells	21(n=12)	17(n=52)

All of the epithelial nuclei of the bronchial tree lie between 17μm and 38μm of the mucociliary surface. Interpreting these figures in terms of the range of alpha particles, we find that a 6.0 MeV-218 Po particle has an effective range of 47μm and a 7.69 MeV-214 Po particle could affect a nucleus up to 70.8μm distant (Lea, 1955).

Gastineau et al, (1972) calculated that over two-thirds of the basal cells in lobar and segmental bronchi (generations 2 - 16) were within range of the 214 Po alpha particle, and about one-fifth were within range of the 218 Po alpha particle; basal cells in the thicker epithelium of mainstem bronchi (generation 1) fell outside of this range. The results of the present study indicate that the nuclei of all basal cells, as well as the nuclei of all other cell types in the bronchial epithelium at all levels are within range of an alpha particle source at the mucociliary surface.

As the total number of airways increases with successive generations, the proximity to each other raises the possibility that epithelial nuclei in one branch may be within range of radioactive particles in another.

Calculations derived from the data of Weibel (Weibel, 1963) and from measurements made by the present authors (Appendix A), reveal that the bronchial tree occupies only a small proportion of the total volume of the lung and that the distance from the mucociliary surface of one conducting airway to the nuclei of the bronchial epithelium of another, exceed the range of alpha particles. However, some interstitial cell nuclei are within range.

The majority of the lungs examined showed scattered small areas of epithelial hyperplasia and squamous metaplasia. The metaplastic changes are of interest because of the frequent occurrence of this change in smokers. Inevitably, this raises the question of a causal relationship between metaplasia and neoplasia. Two factors may be considered: the lack of a protective mucociliary layer and the thickness of the altered epithelium. The absence of ciliary movement inhibits clearance, allowing inhaled particles to remain on the surface. This would increase the duration of exposure to radioactivity and it would increase the chances of epithelial penetration by particles. A thin epithelium would magnify these hazards. Figure 26 depicts the mean thickness of the zones of metaplasia and hyperplasia. Given the absence of cilia, all of the nuclei in the metaplastic epithelium would be well within the range of the radioactive particles described above. Furthermore, the range of measurements was large, with some metaplastic areas being very thin indeed (Table 11).

The significance of long term retention of radioactive dust in the lungs of miners is not known. These deposits are concentrated in perivascular and peribronchiolar regions. None of the lungs examined in this study exhibited heavy accumulations of dust, and we recorded only a few measurements of the distances between dust-laden peribronchiolar macrophages and the nuclei of basal epithelial cells. The range of figures, 3.43 - 9.03 μm, suggests that aggregates of radioactive dust in such peribronchiolar sites could, depending upon their half-lives and energies affect the basal cells of the bronchial epithelium.

To this point, we have focused attention upon the role of the basal cell as progenitor of bronchial epithelium. The demonstration of two morphological types of basal cells suggests they may have more than one function. The presence of hemidesmosomes between basal cells and the basement membrane, desmosomes between basal cells, and desmosomes between these cells and the overlying columnar cells, indicates that basal cells play a role in anchorage of epithelial cells. Similar observations have been made in other animals by Evans and Plopper (1987). Since columnar cells do not connect directly with the basement membrane, anchorage is provided indirectly by the basal cells. One other feature of the elongated basal cell may be of functional importance. The ultrastructural features of these cells resemble those found in myoepithelial cells. If these are contractile cells, is it possible that, in addition to providing anchorage, they may be capable of modifying the overall thickness of the bronchial epithelium?

E. TABLES

TABLE 1

PATIENT'S HISTORY

SMOKERS < 25 PACK-YEARS

#	AGE	SEX	OCCUPATION	PACK-YEARS	SMOKING HISTORY CEASED PRIOR TO SURGERY	ANATOMICAL LOCATION *	PATHOLOGIC DIAGNOSIS
21	54	M	CLERK	6	20 YEARS	RP	METASTATIC CELL CARCINOMA
26	70	F	HOUSEWIFE	13		RU	*
5	61	M	FARMER	15		LL	SQUAMOUS CELL CARCINOMA
6	56	F	HOUSEWIFE	23		LU	*

SMOKERS ≥ 25 PACK-YEARS

#	AGE	SEX	OCCUPATION	PACK-YEARS	SMOKING HISTORY CEASED PRIOR TO SURGERY	ANATOMICAL LOCATION *	PATHOLOGIC DIAGNOSIS
25	61	F	HOUSEWIFE	25	1 MONTH	RL	ADENOCARCINOMA
8	50	M	UNKNOWN	28		RU	NON-SPECIFIC INFLAMMATORY CHANGE
17	58	F	RETAIL SALES	29		LL	ADENOCARCINOMA
27	63	F	HOUSEWIFE	30		RP	ADENOCARCINOMA
24	50	F	HOUSEWIFE	36		RM & RL	SQUAMOUS CELL CARCINOMA
22	50	F	HOUSEWIFE	37	4 YEARS	RU	LARGE CELL CARCINOMA
13	57	M	FISHERMAN/ HUNTER	37		LL	SQUAMOUS CELL CARCINOMA
9	76	F	HOUSEWIFE	40	3 YEARS	RP	LARGE CELL CARCINOMA
14	63	M	REPAIRMAN	47		LP	SQUAMOUS CELL CARCINOMA

SMOKERS ≥ 50 PACK-YEARS

#	AGE	SEX	OCCUPATION	PACK-YEARS	SMOKING HISTORY CEASED PRIOR TO SURGERY	ANATOMICAL LOCATION *	PATHOLOGIC DIAGNOSIS
7	68	F	CLERK/COOK	50		LU	SQUAMOUS CELL CARCINOMA
19	73	M	CARPENTER	50		LU	*
15	67	M	MECHANIC	54		RM & RL	*
18	46	M	MECHANIC	55		LL	HAMARTOMA
20	49	M	LABORER	60		LP	SQUAMOUS CELL CARCINOMA
3	63	M	LABORER	60		LP	ADENOCARCINOMA
30	70	M	MECHANIC/FARMER	60	MONTHS	RL	SQUAMOUS CELL CARCINOMA
4	77	M	FARMER/ RAILWAY WORKER	60		RL	SQUAMOUS CELL CARCINOMA
16	67	M	ENGINEER/ FIREMAN	74	4 YEARS	RM & RL	ADENOCARCINOMA
23	71	M	FARMER	74		RU	SQUAMOUS CELL CARCINOMA
28	62	M	FARMER	94	7 YEARS	RU	ADENOCARCINOMA

SMOKERS ≥ 100 PACK-YEARS

#	AGE	SEX	OCCUPATION	PACK-YEARS	SMOKING HISTORY CEASED PRIOR TO SURGERY	ANATOMICAL LOCATION *	PATHOLOGIC DIAGNOSIS
12	60	M	SALESMAN	100		LU	SQUAMOUS CELL CARCINOMA
11	64	M	CAB DRIVER	150	4 YEARS	LU	*

NON-SMOKERS

#	AGE	SEX	OCCUPATION	PACK-YEARS	SMOKING HISTORY CEASED PRIOR TO SURGERY	ANATOMICAL LOCATION *	PATHOLOGIC DIAGNOSIS
2	65	M	MACHINE OPERATOR			RL	SQUAMOUS CELL CARCINOMA
31	31	F	TEACHER			LL	METASTATIC CARCINOMA
29	39	F	OFFICE WORKER			RL	BRONCHIOLO-ALVEOLAR CARCINOMA

* LP LEFT LUNG LL LEFT LOWER LOBE LU LEFT UPPER LOBE
RP RIGHT LUNG RL RIGHT LOWER LOBE RU RIGHT UPPER LOBE
RM RIGHT MIDDLE LOBE

TABLE 2

NUMBER OF BRONCHI AND CELLS MEASURED
(CELLS IN PARENTHESES) PER CASE

Bronchial Size	≥ 9.01 mm	7.01-9 mm	5.51-7 mm	4.51-5.5 mm	2.01-4.5 mm	0.01-2 mm	Total
Weibel Generation	1	2	3	4	5-7	7-10	
Case # 2	5(321)		1(40)	4(125)	6(177)	2(81)	18(744)
3	2(91)	5(178)	3(61)	2(93)	3(111)	5(98)	21(636)
4					3(99)	6(93)	9(192)
5			2(23)	2(25)	2(14)	3(68)	9(130)
6				2(48)	7(82)	4(49)	13(179)
7				6(61)	5(39)		11(100)
8				1(13)	2(16)		3(29)
9						2(22)	2(22)
11					12(185)		12(185)
12	3(111)	1(39)	3(44)	4(154)	14(485)	2(84)	27(917)
13	6(343)		6(332)	9(252)	7(223)	4(92)	32(1242)
14			5(219)	2(97)	13(586)	2(130)	22(1032)
15			3(40)	2(115)	18(512)	14(247)	37(914)
16	2(47)		1(46)		21(543)	1(22)	25(658)
17					5(112)	2(6)	7(118)
18	2(51)		2(31)	2(40)	3(109)	1(32)	10(263)
19	2(22)			1(21)	12(133)	3(123)	17(299)
20	3(156)			2(111)	4(154)	2(63)	11(484)
21	1(73)	1(32)	6(304)	18(874)	6(110)		32(1393)
22			1(21)		8(233)	10(279)	19(533)
23			1(46)	3(65)	12(344)	2(71)	18(526)
24				5(185)	8(474)	3(112)	16(771)
25	1(32)		2(32)		4(97)	1(19)	8(180)
27	2(24)		2(77)		9(179)	6(117)	19(396)
28			1(31)	1(31)	6(324)	5(162)	13(548)
29	1(57)	1(114)		1(57)	5(281)	1(46)	9(555)
30	1(80)				5(279)		6(343)
31		1(29)		3(110)	8(311)	7(234)	19(684)

TABLE 3

BRONCHI AND CELLS MEASURED BY BRONCHIAL GENERATION

Bronchial Size in mm.	≥ 9.01	7.01 - 9	5.51 - 7	4.51 - 5.5	2.01 - 4.5	0.26 -2
Weibel Generation	1	2	3	4	5 - 7	7 - 16
Bronchi Measured	31	9	39	70	207	88
Cells Measured	1393	392	1347	2487	6532	2250
AVERAGE	44.93	43.55	34.53	35.52	31.55	25.56

TABLE 4

Average Number of Bronchi Measured per Case

$$\frac{444}{28} \quad \begin{matrix}\text{Bronchi} \\ \text{Cases}\end{matrix} \qquad 15.85$$

Average Number of Cells Measured per Bronchus

$$\frac{14401}{444} \quad \begin{matrix}\text{Cells} \\ \text{Bronchi}\end{matrix} \qquad 32.43$$

SMOKERS

Bronchi Measured	398
Cells Measured	12405
Average Cells/Bronchus	31.16

NON-SMOKERS

Bronchi Measured	46
Cells Measured	1996
Average Cells/Bronchus	43.39

TABLE: 5A

EPITHELIAL MEASUREMENTS (μM)

AIRWAY GENERATION 1

AIRWAY DIAMETER ≥ 9.01 mm

CELL TYPE		< 25 PACK YEARS	25-49 PACK YEARS	50-99 PACK YEARS	> 100 PACK YEARS	NON-SMOKERS	P VALUES
CILIATED	A*	-	28.19 ± 4.66	33.29 ± 3.42	32.74	24.93	0.7036
	B	-	34.55 ± 5.32	40.04 ± 3.66	38.67	31.22	0.7199
	C	-	39.28 ± 4.61	43.84 ± 4.08	44.26	35.05	
NON-CILIATED	A	-	34.33	-	-	24.80	**
	B	-	34.33	-	-	24.80	"
	C	-	37.66	-	-	28.97	"
GOBLET	A	-	28.72 ± 7.78	35.29 ± 2.91	31.07	19.88	0.3770
	B	-	28.72 ± 7.78	35.29 ± 2.91	31.07	19.88	0.3770
	C	-	32.79 ± 7.93	40.16 ± 3.43	43.94	24.72	0.3687
BASAL TYPICAL	A	-	28.50 ± 5.65	37.55 ± 4.03	32.48	27.14	0.5515
	B	-	35.19 ± 6.00	44.48 ± 4.21	38.90	32.75	0.5361
	C	-	40.99 ± 4.31	48.47 ± 4.70	44.76	35.91	0.5935
BASAL ATYPICAL	A	-	24.91 ± 3.73	28.70 ± 4.47	36.68	21.42	0.5200
	B	-	30.60 ± 4.51	34.45 ± 4.54	41.85	27.80	0.5948
	C	-	33.89 ± 3.45	39.06 ± 6.25	47.93	30.46	0.5941

*A = APEX OF CELL TO EPITHELIAL BASEMENT MEMBRANE
B = A + CILIA
C = B + BASEMENT MEMBRANE THICKNESS

**INSUFFICIENT DATA FOR ANALYSIS

NUMBERS REPRESENT A MEANS ± STANDARD ERROR

---

1 ABSENCE OF STANDARD ERROR IS DUE TO INSUFFICIENT NUMBER OF CASES.

2 P VALUES = COMPARISONS OF ALL COLUMNS.

TABLE: 5B

EPITHELIAL MEASUREMENTS (μM)

AIRWAY GENERATION 2

AIRWAY DIAMETER 7.01 - 9.00 mm

CELL TYPE		< 25 PACK YEARS	25-49 PACK YEARS	50-99 PACK YEARS	> 100 PACK YEARS	NON-SMOKERS	P VALUES
CILIATED	A*	14.19	-	34.82	38.04	36.44 ± 6.08	**
	B	19.84	-	41.68	44.88	42.78 ± 4.99	"
	C	22.48	-	44.30	49.92	48.43 ± 3.99	"
NON-CILIATED	A	-	-	35.80	-	30.01	"
	B	-	-	35.80	-	30.01	"
	C	-	-	38.15	-	32.17	"
GOBLET	A	17.78	-	36.66	-	36.26	"
	B	17.78	-	36.66	-	36.26	"
	C	20.40	-	41.15	-	41.05	"
BASAL TYPICAL	A	15.46	-	35.35	40.12	36.10 ± 4.41	"
	B	21.01	-	42.48	47.71	41.70 ± 4.06	"
	C	23.57	-	45.17	53.59	47.01 ± 3.51	"
BASAL ATYPICAL	A	16.05	-	36.16	-	24.13 ± 7.65	"
	B	21.80	-	43.55	-	29.33 ± 6.77	"
	C	24.32	-	45.57	-	31.88 ± 5.60	"

*A = APEX OF CELL TO EPITHELIAL BASEMENT MEMBRANE
B = A + CILIA
C = B + BASEMENT MEMBRANE THICKNESS

**INSUFFICIENT DATA FOR ANALYSIS

NUMBERS REPRESENT A MEANS ± STANDARD ERROR

---

1 ABSENCE OF STANDARD ERROR IS DUE TO INSUFFICIENT NUMBER OF CASES.

2 P VALUES = COMPARISONS OF ALL COLUMNS.

TABLE: 5C

EPITHELIAL MEASUREMENTS (μM)

AIRWAY GENERATION 3

AIRWAY DIAMETER 5.51 - 7.00 mm

CELL TYPE		< 25 PACK YEARS	25-49 PACK YEARS	50-99 PACK YEARS	> 100 PACK YEARS	NON-SMOKERS	P VALUES
CILIATED	A*	36.00 ± 17.88	25.15 ± 3.09	26.00 ± 2.72	42.30	28.48	0.5852
	B	42.53 ± 17.93	31.42 ± 3.04	32.22 ± 2.92	48.67	34.31	0.5832
	C	45.36 ± 18.17	35.45 ± 3.31	36.29 ± 2.88	55.12	36.88	0.5504
NON-CILIATED	A	-	23.33 ± 3.39	20.47 ± 5.57	34.13	-	0.4162
	B	-	23.33 ± 3.39	20.47 ± 5.57	34.13	-	0.4162
	C	-	27.93 ± 2.82	24.65 ± 7.43	44.33	-	0.3157
GOBLET	A	40.40 ± 14.82	27.75 ± 3.69	28.64 ± 1.61	43.74	-	0.3393
	B	40.40 ± 14.82	27.75 ± 3.69	28.64 ± 1.61	43.74	-	0.3393
	C	44.67 ± 15.36	33.11 ± 4.01	33.99 ± 1.85	47.47	-	0.4498
BASAL TYPICAL	A	37.52 ± 11.43	26.74 ± 2.48	28.60 ± 3.95	35.42	29.74	0.7218
	B	44.04 ± 10.50	32.62 ± 2.66	34.38 ± 3.96	41.72	35.95	0.6554
	C	48.06 ± 11.38	37.00 ± 2.60	38.11 ± 4.03	50.52	38.13	0.5612
BASAL ATYPICAL	A	21.38	21.64 ± 3.03	20.22 ± 2.16	-	-	0.9210
	B	29.72	27.70 ± 2.66	25.56 ± 1.94	-	-	0.6410
	C	32.86	31.37 ± 3.20	29.29 ± 1.72	-	-	0.7180

*A = APEX OF CELL TO EPITHELIAL BASEMENT MEMBRANE
B = A + CILIA
C = B + BASEMENT MEMBRANE THICKNESS

**INSUFFICIENT DATA FOR ANALYSIS

NUMBERS REPRESENT A MEANS ± STANDARD ERROR

---

1 ABSENCE OF STANDARD ERROR IS DUE TO INSUFFICIENT NUMBER OF CASES.

2 P VALUES = COMPARISONS OF ALL COLUMNS.

TABLE: 5D

EPITHELIAL MEASUREMENTS (μM)

AIRWAY GENERATION 4

AIRWAY DIAMETER 4.51 - 5.50 mm

CELL TYPE		< 25 PACK YEARS	25-49 PACK YEARS	50-99 PACK YEARS	> 100 PACK YEARS	NON-SMOKERS	P VALUES
CILIATED	A*	33.36 ± 15.30	25.19 ± 4.62	22.48 ± 2.47	28.93	25.51 ± 2.46	0.6601
	B	39.27 ± 15.37	31.98 ± 4.80	28.32 ± 2.64	34.90	31.23 ± 2.45	0.6734
	C	43.26 ± 16.02	35.63 ± 5.77	31.21 ± 2.91	40.52	35.55 ± 3.23	0.6358
NON-CILIATED	A	22.76 ± 0.79	23.92 ± 3.47	26.16 ± 3.85	25.11	27.60 ± 1.51	0.8372
	B	22.76 ± 0.79	23.92 ± 3.47	26.16 ± 3.85	25.11	27.60 ± 1.51	0.8372
	C	27.17 ± 1.28	27.66 ± 3.51	30.73 ± 6.55	29.33	31.25 ± 3.40	0.9336
GOBLET	A	40.92 ± 7.67	26.31 ± 3.93	28.83 ± 0.89	-	26.16 ± 2.87	0.0843
	B	40.92 ± 7.67	26.31 ± 3.93	28.83 ± 0.89	-	26.16 ± 2.87	0.0843
	C	47.45 ± 8.82	30.22 ± 4.87	32.83 ± 1.14	-	31.33 ± 3.35	0.0858
BASAL TYPICAL	A	30.36 ±10.06	26.89 ± 4.90	26.59 ± 1.60	33.41	28.12 ± 2.19	0.8647
	B	36.16 ±10.24	33.49 ± 4.78	32.62 ± 1.51	39.74	33.95 ± 2.44	0.8701
	C	39.54 ± 9.80	37.02 ± 5.72	36.48 ± 1.84	45.49	33.20 ± 2.79	0.8305
BASAL ATYPICAL	A	17.56	21.17 ± 3.50	21.24 ± 1.99	26.38	24.41 ± 1.45	0.6589
	B	22.75	26.86 ± 3.08	26.13 ± 2.03	32.42	29.51 ± 1.55	0.5910
	C	25.64	29.68 ± 3.84	28.77 ± 2.33	37.17	33.25 ± 2.16	0.5435

*A = APEX OF CELL TO EPITHELIAL BASEMENT MEMBRANE
B = A + CILIA
C = B + BASEMENT MEMBRANE THICKNESS

**INSUFFICIENT DATA FOR ANALYSIS

NUMBERS REPRESENT A MEANS ± STANDARD ERROR

---

1 ABSENCE OF STANDARD ERROR IS DUE TO INSUFFICIENT NUMBER OF CASES.

2 P VALUES = COMPARISONS OF ALL COLUMNS.

TABLE: 5E

EPITHELIAL MEASUREMENTS (μM)

AIRWAY GENERATION 5 - 7

AIRWAY DIAMETER 2.01 - 4.50 mm

CELL TYPE		< 25 PACK YEARS	25-49 PACK YEARS	50-99 PACK YEARS	> 100 PACK YEARS	NON-SMOKERS	P VALUES
CILIATED	A*	30.41 ± 6.37	26.46 ± 2.74	28.68 ± 2.96	28.56 ± 1.54	20.67 ± 1.01	0.6323
	B	35.97 ±10.87	32.29 ± 2.70	34.49 ± 2.97	34.64 ± 1.80	26.32 ± 1.02	0.6225
	C	39.83 ± 6.80	35.87 ± 2.66	39.31 ± 2.98	39.29 ± 2.88	30.35 ± 1.52	0.5591
NON-CILIATED	A	14.52	20.10 ± 2.43	24.82 ± 2.93	22.21 ± 5.93	16.44 ± 0.91	0.3748
	B	14.52	20.10 ± 2.43	24.82 ± 2.93	22.21 ± 5.93	16.44 ± 0.91	0.3748
	C	18.73	23.34 ± 2.24	27.98 ± 2.94	26.17 ± 7.54	20.34 ± 0.81	0.4776
GOBLET	A	39.19 ± 8.95	29.85 ± 3.78	31.68 ± 2.86	33.45 ± 2.17	23.56 ± 1.74	0.4176
	B	39.19 ± 8.95	29.85 ± 3.78	31.68 ± 2.86	33.45 ± 2.17	23.56 ± 1.74	0.4176
	C	44.82 ±10.42	34.26 ± 3.97	36.16 ± 3.06	38.70 ± 2.96	28.29 ± 2.37	0.4385
BASAL TYPICAL	A	41.54 ±11.01	29.83 ± 3.67	31.35 ± 2.57	30.31 ± 1.03	22.61 ± 2.01	0.2587
	B	47.39 ±11.18	35.01 ± 3.11	37.15 ± 2.59	36.79 ± 1.22	27.84 ± 1.84	0.1996
	C	51.67 ±11.62	38.77 ± 2.77	41.33 ± 2.81	41.23 ± 2.12	31.68 ± 2.33	0.2024
BASAL ATYPICAL	A	24.85 ± 7.13	21.61 ± 2.62	22.05 ± 1.42	24.13 ± 1.55	18.65 ± 0.32	0.7374
	B	31.07 ± 7.96	26.80 ± 2.86	27.39 ± 1.40	29.88 ± 1.85	23.60 ± 0.16	0.6582
	C	33.38 ± 7.62	29.87 ± 2.96	30.55 ± 1.51	33.96 ± 2.61	27.39 ± 0.34	0.7419

*A = APEX OF CELL TO EPITHELIAL BASEMENT MEMBRANE
B = A + CILIA
C = B + BASEMENT MEMBRANE THICKNESS

**INSUFFICIENT DATA FOR ANALYSIS

NUMBERS REPRESENT A MEANS ± STANDARD ERROR

---

1 ABSENCE OF STANDARD ERROR IS DUE TO INSUFFICIENT NUMBER OF CASES.

2 P VALUES = COMPARISONS OF ALL COLUMNS.

TABLE: 5F

EPITHELIAL MEASUREMENTS (μM)

AIRWAY GENERATION 7 - 16

AIRWAY DIAMETER 0.26 - 2.00 mm

CELL TYPE		< 25 PACK YEARS	25-49 PACK YEARS	50-99 PACK YEARS	> 100 PACK YEARS	NON-SMOKERS	P VALUES
CILIATED	A*	14.92 ± 1.48	21.93 ± 3.29	23.10 ± 1.60	18.12	17.73 ± 0.75	0.4094
	B	19.61 ± 1.30	10.21 ± 3.40	28.84 ± 1.67	23.71	23.10 ± 0.86	0.3410
	C	20.89 ± 1.45	30.63 ± 3.55	31.51 ± 1.61	28.65	25.94 ± 1.11	0.2651
NON-CILIATED	A	11.59 ± 0.11	19.95 ± 3.04	19.95 ± 1.29	16.28	13.81 ± 2.73	0.0862
	B	11.59 ± 0.11	19.67 ± 3.04	19.95 ± 1.29	16.28	13.81 ± 2.73	0.0862
	C	12.16 ± 0.68	25.38 ± 5.49	23.32 ± 1.46	20.92	16.07 ± 3.32	0.1036
GOBLET	A	25.71 ±11.00	28.04 ± 5.73	29.05 ± 4.43	-	19.77 ± 4.20	0.7805
	B	25.71 ±11.00	28.04 ± 5.73	29.05 ± 4.43	-	19.77 ± 4.20	0.7805
	C	29.49 ±11.79	32.47 ± 6.04	32.24 ± 4.75	-	23.10 ± 3.96	0.7972
BASAL TYPICAL	A	17.98 ± 3.01	-	30.57 ± 1.78	-	18.60	0.0517
	B	22.92 ± 2.19	-	36.95 ± 1.74	-	23.91	0.0269
	C	23.56 ± 1.88	-	39.34 ± 1.47	-	25.76	0.0130
BASAL ATYPICAL	A	12.94 ± 2.85	20.13 ± 2.67	22.39 ± 1.53	17.56	16.90 ± 2.19	0.2654
	B	17.94 ± 2.84	25.91 ± 2.85	28.04 ± 1.69	23.01	22.15 ± 2.33	0.2642
	C	19.37 ± 3.13	28.83 ± 2.89	30.57 ± 1.47	28.09	24.37 ± 2.77	0.1878

*A = APEX OF CELL TO EPITHELIAL BASEMENT MEMBRANE
B = A + CILIA
C = B + BASEMENT MEMBRANE THICKNESS

**INSUFFICIENT DATA FOR ANALYSIS

NUMBERS REPRESENT A MEANS ± STANDARD ERROR

---

1 ABSENCE OF STANDARD ERROR IS DUE TO INSUFFICIENT NUMBER OF CASES.

2 P VALUES = COMPARISONS OF ALL COLUMNS.

TABLE: 6

EPITHELIAL THICKNESS (μm)

AIRWAY GENERATION		1	2	3	4	5 - 7	7 - 16	P VALUES
AIRWAY DIAMETER		≥9.01	7.01 - 9	5.51 - 7	4.51 - 5.5	2.01 - 4.5	0.26 - 2	
CELL TYPE								
CILIATED	A*	31.17±2.23	31.98±3.03	29.02±3.03	25.32±2.13	27.35±1.63	20.94±1.32	0.0051
	B	37.69±2.40	38.39±4.93	35.28±3.07	31.33±2.18	33.14±1.62	26.49±1.44	0.0024
	C	41.89±2.53	42.71±5.30	39.21±3.15	34.94±2.42	37.41±1.67	29.23±1.50	0.0006
NON-CILIATED	A	29.57±4.77	32.91±2.90	23.65±3.07	24.99±1.24	21.51±1.67	17.76±1.19	0.0038
	B	29.57±4.77	32.91±2.90	23.65±3.07	24.99±1.24	21.51±1.67	17.76±1.19	0.0038
	C	33.32±4.35	35.16±2.99	28.87±4.00	29.06±1.54	24.96±1.67	21.12±1.68	0.0137
GOBLET	A	31.65±2.67	30.23±6.23	32.24±3.21	30.76±2.30	31.25±1.92	26.96±2.77	0.7801
	B	31.65±2.67	30.23±6.23	32.24±3.21	30.75±2.30	31.25±1.92	26.96±2.77	0.7801
	C	37.22±2.99	34.20±6.90	37.21±3.19	35.55±2.68	35.94±2.09	30.60±2.94	0.6274
BASAL TYPICAL	A	33.35±2.73	32.62±4.59	30.28±2.45	27.91±1.55	31.01±2.00	23.15±1.13	0.0052
	B	39.99±2.88	38.92±4.79	36.39±2.44	34.01±1.55	36.65±1.97	28.69±1.17	0.0026
	C	44.57±2.88	43.29±5.25	40.52±2.63	37.94±1.71	40.71±2.03	31.39±1.26	0.0005
BASAL ATYPICAL	A	27.72±2.61	25.12±5.19	20.90±1.43	21.96±1.22	21.90±1.09	19.59±1.27	0.0396
	B	33.46±2.61	31.00±5.32	26.88±1.37	27.15±1.23	27.25±1.18	25.15±1.37	0.0461
	C	37.62±3.27	33.41±4.98	30.52±1.41	30.19±1.48	30.47±1.21	27.78±1.40	0.0259

*A = APEX OF CELL TO EPITHELIAL BASEMENT MEMBRANE
B = A + CILIA
C = B + BASEMENT MEMBRANE THICKNESS

NUMBERS REPRESENT MEANS ± STANDARD ERROR

[1] P VALUES = COMPARISONS OF ALL COLUMNS.

TABLE 6.1

ANALYSIS OF VARIANCE BETWEEN INDIVIDUAL BRONCHIAL GENERATION

Cell Type		Gen 1+2	Gen 1+3	Gen 1+4	Gen 1+5-7	Gen 1+7-16	Gen 2+3	Gen 2+4	Gen 2+5-7	Gen 2+7-16	Gen 3+4	Gen 3+5-7	Gen 3+7-16	Gen 4+5-7	Gen 4+7-16	Gen 5-7 & 7-16
CILIATED	A***	0.8606*	0.5591	0.0946	0.2375	0.0020**	0.5044	0.1209	0.2561	0.0078**	0.2464	0.5454	0.0065**	0.4442	0.1009	0.0067**
	B	0.8825	0.5226	0.0760	0.1693	0.0010**	0.4930	0.1085	0.2085	0.0051**	0.2266	0.4706	0.0039**	0.5063	0.0760	0.0059**
	C	0.8699	0.4973	0.0649	0.1970	0.0004**	0.4632	0.0928	0.2260	0.0026**	0.2136	0.5630	0.0019**	0.3870	0.0469**	0.0014**
NON-CILIATED	A	0.5818	0.2265	0.3317	0.0792	0.0122**	0.0612	0.0963	0.0145**	0.0016**	0.6533	0.4302	0.0377**	0.1499	0.0050**	0.0808
	B	0.5818	0.2265	0.3317	0.0792	0.0122**	0.0612	0.0963	0.0145**	0.0016**	0.6533	0.4302	0.0377**	0.1499	0.0050**	0.0808
	C	0.7948	0.4354	0.4398	0.1185	0.0260**	0.2713	0.2696	0.0582	0.0110**	0.9562	0.2195	0.0203**	0.1470	0.0082**	0.1259
TYPICAL BASAL	A	0.8740	0.4054	0.1260	0.4767	0.0026**	0.5861	0.2597	0.6857	0.0188**	0.4484	0.7914	0.0141**	0.2353	0.0759	0.0089**
	B	0.8163	0.3188	0.0944	0.3096	0.0010**	0.5432	0.2424	0.5673	0.0118**	0.4670	0.8989	0.0093**	0.3154	0.0489**	0.0008**
	C	0.7931	0.3015	0.0793	0.2659	0.0003**	0.5437	0.2277	0.5370	0.0057**	0.4362	0.9504	0.0032**	0.3194	0.0219**	0.0002**
ATYPICAL BASAL	A	0.4636	0.0221**	0.0285**	0.0193**	0.0016**	0.2251	0.3219	0.2947	0.0772	0.6690	0.6669	0.5798	0.9747	0.2222	0.1841
	B	0.5071	0.0342**	0.0219**	0.0170**	0.0020**	0.2568	0.2483	0.2436	0.0735	0.9184	0.8784	0.4832	0.9560	0.3731	0.2457
	C	0.2882	0.0321**	0.0118**	0.0103**	0.0007**	0.4541	0.3650	0.3905	0.1061	0.9056	0.9863	0.3014	0.8925	0.2654	0.1655

*P VALUES
**SIGNIFICANT P VALUES ($p<0.05$)
***A = APEX OF CELL TO EPITHELIAL BASEMENT MEMBRANE
B = A + CILIA
C = B + BASEMENT MEMBRANE THICKNESS

TABLE 6.2A

STATISTICALLY SIGNIFICANT DIFFERENCES OF EPITHELIAL MEASUREMENTS FOR ALL BRONCHIAL GENERATIONS

CILIATED CELLS

A (APEX OF CELL TO EPITHELIAL BASEMENT MEMBRANE)

SIGNIFICANCE FOUND BETWEEN

GENERATION	1 & 7-16	p =	0.0026
	2 & 7-16	p =	0.0078
	3 & 7-16	p =	0.0065
	5-7 & 7-16	p =	0.0067

B (A + CILIA)

SIGNIFICANCE FOUND BETWEEN

GENERATION	1 & 7-16	p =	0.0010
	2 & 7-16	p =	0.0051
	3 & 7-16	p =	0.0039
	5-7 & 7-16	p =	0.0059

C (B + BASEMENT MEMBRANE THICKNESS)

SIGNIFICANCE FOUND BETWEEN

GENERATION	1 & 7-16	p =	0.0004
	2 & 7-16	p =	0.0026
	3 & 7-16	p =	0.0019
	4 & 7-16	p =	0.0469
	5-7 & 7-16	p =	0.0014

TABLE 6.2B

STATISTICALLY SIGNIFICANT DIFFERENCES OF EPITHELIAL MEASUREMENTS FOR ALL BRONCHIAL GENERATIONS

NON-CILIATED CELLS

A (APEX OF CELL TO EPITHELIAL BASEMENT MEMBRANE)

SIGNIFICANCE FOUND BETWEEN

GENERATION	1 & 7-16	p =	0.0026
	2 & 7-16	p =	0.0078
	3 & 7-16	p =	0.0065
	5-7 & 7-16	p =	0.0067

B (A + CILIA)

SIGNIFICANCE FOUND BETWEEN

GENERATION	1 & 7-16	p =	0.0122
	2 & 5-7	p =	0.0145
	2 & 7-16	p =	0.0016
	3 & 7-16	p =	0.0377
	4 & 7-16	p =	0.0050

C (B + BASEMENT MEMBRANE THICKNESS)

SIGNIFICANCE FOUND BETWEEN

GENERATION	1 & 7-16	p =	0.0260
	2 & 7-16	p =	0.0010
	3 & 7-16	p =	0.0203
	4 & 7-16	p =	0.0082

TABLE 6.2C

STATISTICALLY SIGNIFICANT DIFFERENCES OF EPITHELIAL MEASUREMENTS FOR ALL BRONCHIAL GENERATIONS

BASAL CELLS - TYPICAL

A (APEX OF CELL TO EPITHELIAL BASEMENT MEMBRANE)

SIGNIFICANT DIFFERENCES FOUND BETWEEN

GENERATION	1 & 7-16	p =	0.0026
	2 & 7-16	p =	0.0188
	3 & 7-16	p =	0.0141
	5-7 & 7-16	p =	0.0009

B (A + CILIA)

SIGNIFICANT DIFFERENCES FOUND BETWEEN

GENERATION	1 & 7-16	p =	0.0010
	2 & 7-16	p =	0.0118
	3 & 7-16	p =	0.0093
	4 & 7-16	p =	0.0489
	5-7 & 7-16	p =	0.0008

C (B + BASEMENT MEMBRANE THICKNESS)

SIGNIFICANT DIFFERENCES FOUND BETWEEN

GENERATION	1 & 7-16	p =	0.0003
	2 & 7-16	p =	0.0057
	3 & 7-16	p =	0.0032
	4 & 7-16	p =	0.0219
	5-7 & 7-16	p =	0.0002

TABLE 6.2D

STATISTICALLY SIGNIFICANT DIFFERENCES OF EPITHELIAL MEASUREMENTS FOR ALL BRONCHIAL GENERATIONS

BASAL CELLS - ATYPICAL

A (APEX OF CEL TO EPITHELIAL BASEMENT MEMBRANE)

SIGNIFICANT DIFFERENCES FOUND BETWEEN

GENERATION	1 & 3	p	=	0.0221
	1 & 4	p	=	0.0285
	1 & 5-7	p	=	0.0193

B (A + CILIA)

SIGNIFICANT DIFFERENCES FOUND BETWEEN

GENERATION	1 & 3	p	=	0.0342
	1 & 4	p	=	0.0219
	1 & 5-7	p	=	0.0170
	1 & 7-16	p	=	0.0020

C (B + BASEMENT MEMBRANE THICKNESS)

SIGNIFICANT DIFFERENCES FOUND BETWEEN

GENERATION	1 & 3	p	=	0.0321
	1 & 4	p	=	0.0118
	1 & 5-7	p	=	0.0103
	1 & 7-16	p	=	0.0007

TABLE: 7A

EPITHELIAL MEASUREMENTS (μM)

CILIATED CELLS

CELL TYPES	GEN 1 ≥ 9.01 mm	GEN 2 7.01 - 9.00 mm	GEN 3 5.51 - 7.00 mm	GEN 4 4.51 - 5.50 mm	GEN 5-7 2.07 - 4.5 mm	GEN 7-16 0.26 - 2.00 mm	P VALUE
CILIA	6.52 ± 0.71	6.40 ± 0.41	6.29 ± 0.21	6.02 ± 0.18	5.84 ± 0.13	5.58 ± 0.12	0.0029
APEX OF CELL TO NUCLEUS	12.86 ± 1.42	10.96 ± 1.79	9.42 ± 0.99	8.61 ± 0.75	9.14 ± 0.60	6.53 ± 0.52	0.0000
NUCLEUS	8.77 ± 0.32	9.57 ± 0.88	9.12 ± 0.53	8.24 ± 0.35	8.58 ± 0.34	8.06 ± 0.31	0.2457
APEX TO EPITHELIAL BASEMENT MEMBRANE	31.17 ± 2.23	31.98 ± 4.87	29.02 ± 3.03	25.32 ± 2.13	27.35 ± 1.63	20.94 ± 1.37	0.0051
CILIA TO EPITHELIAL BASEMENT MEMBRANE	37.69 ± 2.40	38.39 ± 4.93	35.78. ± 3.07	31.33 ± 2.18	33.14 ± 1.62	26.49 ± 1.44	0.0024
EPITHELIAL BASEMENT MEMBRANE	4.30 ± 0.43	4.39 ± 0.73	4.13 ± 0.46	3.64 ± 0.33	4.07 ± 0.22	2.87 ± 0.20	0.0035

NUMBERS REPRESENT MEANS ± STANDARD ERROR

TABLE: 7B

EPITHELIAL MEASUREMENTS (μM)

NON-CILIATED CELLS

CELL TYPES	GEN 1 ≥9.01 mm	GEN 2 7.01 - 9.00 mm	GEN 3 5.51 - 7.00 mm	GEN 4 4.51 - 5.50 mm	GEN 5-7 2.07 - 4.5 mm	GEN 7-16 0.26 - 2.00 mm	P VALUE
CILIA	-	-	-	-	-	-	-
APEX OF CELL TO NUCLEUS	10.08 ± 2.49	13.05 ± 1.67	9.10 ± 1.59	9.10 ± 1.09	7.32 ± 0.53	6.41 ± 0.59	0.0167
NUCLEUS	9.63 ± 0.10	7.85 ± 0.40	7.91 ± 0.56	8.21 ± 0.37	6.42 ± 0.36	6.75 ± 0.35	0.0031
APEX TO EPITHELIAL BASEMENT MEMBRANE	29.57 ± 4.77	32.91 ± 2.90	23.65 ± 3.07	24.99 ± 1.24	21.51 ± 1.67	17.76 ± 1.19	0.0038
CILIA TO EPITHELIAL BASEMENT MEMBRANE	29.57 ± 4.77	32.91 ± 2.90	23.65 ± 3.07	24.99 ± 1.24	21.51 ± 1.67	17.76 ± 1.19	0.0038
EPITHELIAL BASEMENT MEMBRANE	3.76 ± 0.43	2.26 ± 0.10	5.22 ± 1.23	4.07 ± 0.61	3.49 ± 0.21	3.41 ± 0.64	0.3472

NUMBERS REPRESENT MEANS ± STANDARD ERROR

TABLE: 7C

EPITHELIAL MEASUREMENTS (μM)

GOBLET CELLS

CELL TYPES	GEN 1 ≥9.01 mm	GEN 2 7.01 - 9.00 mm	GEN 3 5.51 - 7.00 mm	GEN 4 4.51 - 5.50 mm	GEN 5-7 2.07 - 4.5 mm	GEN 7-16 0.26 - 2.00 mm	P VALUE
CILIA	-	-	-	-	-	-	-
APEX OF CELL TO NUCLEUS	19.83 ± 1.77	18.42 ± 3.05	19.42 ± 1.39	19.37 ± 1.58	20.32 ± 1.30	18.11 ± 2.07	0.9430
NUCLEUS	5.52 ± 0.66	5.08 ± 1.28	6.21 ± 0.81	5.65 ± 0.37	5.36 ± 0.32	4.57 ± 0.35	0.3446
APEX TO EPITHELIAL BASEMENT MEMBRANE	31.65 ± 2.67	30.23 ± 6.23	32.24 ± 3.21	30.76 ± 2.30	31.25 ± 1.92	26.96 ± 2.77	0.7801
CILIA TO EPITHELIAL BASEMENT MEMBRANE	31.65 ± 2.67	30.23 ± 6.23	32.24 ± 3.21	30.76 ± 2.30	31.28 ± 1.92	26.96 ± 2.77	0.7787
EPITHELIAL BASEMENT MEMBRANE	5.58 ± 0.97	3.96 ± 0.68	4.97 ± 0.41	4.79 ± 0.48	4.64 ± 0.29	3.65 ± 0.28	0.1565

NUMBERS REPRESENT MEANS ± STANDARD ERROR

TABLE: 7D

EPITHELIAL MEASUREMENTS (μM)

TYPICAL BASAL CELLS

CELL TYPES	GEN 1 ≥ 9.01 mm	GEN 2 7.01 - 9.00 mm	GEN 3 5.51 - 7.00 mm	GEN 4 4.51 - 5.50 mm	GEN 5-7 2.07 - 4.5 mm	GEN 7-16 0.26 - 2.00 mm	P VALUE
CILIA	6.85 ± 0.19	6.44 ± 0.45	6.18 ± 0.26	6.40 ± 0.17	5.89 ± 0.27	5.69 ± 0.15	0.0335
APEX OF CELL TO NUCLEUS	26.65 ± 2.36	25.83 ± 3.96	24.08 ± 2.22	22.02 ± 1.29	24.59 ± 1.76	17.46 ± 0.93	0.0033
NUCLEUS	4.71 ± 0.28	4.87 ± 0.44	4.54 ± 0.21	4.58 ± 0.20	4.69 ± 0.20	4.26 ± 0.18	0.5415
APEX TO EPITHELIAL BASEMENT MEMBRANE	33.35 ± 2.73	32.62 ± 4.59	30.28 ± 2.45	27.91 ± 1.55	31.03 ± 2.00	23.15 ± 1.13	0.0052
CILIA TO EPITHELIAL BASEMENT MEMBRANE	39.99 ± 2.88	38.92 ± 4.79	36.39 ± 2.44	34.01 ± 1.55	36.65 ± 1.97	28.69 ± 1.17	0.0026
EPITHELIAL BASEMENT MEMBRANE	4.63 ± 0.62	4.39 ± 0.73	4.31 ± 0.62	3.96 ± 0.33	4.07 ± 0.29	2.85 ± 0.26	0.0176

NUMBERS REPRESENT MEANS ± STANDARD ERROR

1 MEASUREMENTS FOR CILIA AND APEX OF CELL TO BASAL CELL NUCLEUS ARE FOR CELLS OVERLYING THE BASAL CELL.

TABLE: 7E

EPITHELIAL MEASUREMENTS (μM)

ATYPICAL BASAL CELLS

CELL TYPES	GEN 1 ≥9.01 mm	GEN 2 7.01 - 9.00 mm	GEN 3 5.51 - 7.00 mm	GEN 4 4.51 - 5.50 mm	GEN 5-7 2.07 - 4.5 mm	GEN 7-16 0.26 - 2.00 mm	P VALUE
CILIA	5.99 ± 0.19	5.89 ± 0.63	6.17 ± 0.45	5.80 ± 0.24	5.79 ± 0.15	5.77 ± 0.19	0.9065
APEX OF CELL TO NUCLEUS	23.34 ± 2.40	21.33 ± 5.07	17.19 ± 1.36	20.11 ± 1.84	18.15 ± 1.08	15.98 ± 1.19	0.0686
NUCLEUS	3.11 ± 0.15	2.96 ± 0.36	2.81 ± 0.15	4.23 ± 1.56	2.83 ± 0.05	2.79 ± 0.09	0.6681
APEX TO EPITHELIAL BASEMENT MEMBRANE	27.72 ± 2.61	25.12 ± 5.19	20.90 ± 1.43	21.96 ± 1.22	21.90 ± 2.09	19.59 ± 1.27	0.0396
CILIA TO EPITHELIAL BASEMENT MEMBRANE	33.46 ± 2.61	31.00 ± 5.32	26.88 ± 1.37	27.15 ± 1.23	27.25 ± 1.18	25.15 ± 1.37	0.0461
EPITHELIAL BASEMENT MEMBRANE	4.59 ± 1.07	2.42 ± 0.50	3.64 ± 0.58	3.07 ± 0.32	3.26 ± 0.20	2.69 ± 0.24	0.0409

NUMBERS REPRESENT MEANS ± STANDARD ERROR

1 MEASUREMENTS FOR CILIA AND APEX OF CELL TO BASAL CELL NUCLEUS ARE FOR CELLS OVERLYING THE BASAL CELL.

TABLE 7.1 - A

ANALYSIS OF VARIANCE BETWEEN INDIVIDUAL BRONCHIAL GENERATIONS.

CELL TYPE - CILIATED	Gen 1+2	Gen 1+3	Gen 1+4	Gen 1+5-7	Gen 1+7-16	Gen 2+3	Gen 2+4	Gen 2+5-7	Gen 2+7-16	Gen 3+4	Gen 3+5-7	Gen 3+7-16	Gen 4+5-7	Gen 4+7-16	Gen 5-7 & 7-16
MEASUREMENTS CILIA	0.7622*	0.4458	0.0778	0.0105**	0.0005**	0.7525	0.2655	0.0884	0.0134**	0.2909	0.0570	0.0030**	0.4024	0.0411**	0.1617
APEX OF CELL TO NUCLEUS	0.2831	0.0156**	0.0017**	0.0030**	0.0000**	0.2588	0.1487	0.2304	0.0056**	0.5054	0.0001	0.0103**	0.6011	0.0407**	0.0037**
APEX OF CELL TO EPITHELIAL BASEMENT MEMBRANE	0.8606	0.5591	0.0966	0.2375	0.0020**	0.5044	0.1209	0.2561	0.0078**	0.2464	0.5654	0.0065**	0.4442	0.1009	0.0067**
CILIA TO EPITHELIAL BASEMENT MEMBRANE	0.6875	0.5236	0.0760	0.1693	0.0010**	0.4930	0.1085	0.2085	0.0051**	0.2366	0.4786	0.0039**	0.5063	0.0760	0.0059**
EPITHELIAL BASEMENT MEMBRANE	0.8934	0.7551	0.2060	0.6260	0.0027**	0.6901	0.2610	0.5969	0.0136**	0.3075	0.8948	0.0045**	0.2796	0.0526	0.0007**

*P VALUES
**SIGNIFICANT P VALUES (p < 0.05)

TABLE 7.1 - B

ANALYSIS OF VARIANCE BETWEEN INDIVIDUAL BRONCHIAL GENERATIONS.

CELL TYPE - NON-CILIATED	Gen 1+2	Gen 1+3	Gen 1+4	Gen 1+5-7	Gen 1+7-16	Gen 2+3	Gen 2+4	Gen 2+5-7	Gen 2+7-16	Gen 3+4	Gen 3+5-7	Gen 3+7-16	Gen 4+5-7	Gen 4+7-16	Gen 5-7 & 7-16
MEASUREMENTS CILIA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
APEX OF CELL TO NUCLEUS	0.3040	0.6694	0.6592	0.2011	0.0937	0.0909	0.0806	0.0098**	0.0033**	0.9990	0.1692	0.0448 **	0.1211	0.0251**	0.3627
CELL TO NUCLEUS EPITHELIAL BASEMENT MEMBRANE	0.5018	0.2265	0.3317	0.0792	0.0122**	0.0612	0.0963	0.0145**	0.0016**	0.6533	0.4302	0.0377**	0.1499	0.0050**	0.0808
CILIA TO EPITHELIAL BASEMENT MEMBRANE	0.5018	0.2265	0.3317	0.0792	0.0122**	0.0612	0.0963	0.0145**	0.0016**	0.6533	0.4302	0.0377**	0.1499	0.0050**	0.0808
EPITHELIAL BASEMENT MEMBRANE	0.4625	0.3714	0.8412	0.8594	0.0226	0.0741	0.2527	0.4188	0.4519	0.2553	0.0605	0.0569	0.4676	0.4289	0.9163

*P VALUES
**SIGNIFICANT p VALUES ($p < 0.05$)

TABLE 7.1 - C

ANALYSIS OF VARIANCE BETWEEN INDIVIDUAL BRONCHIAL GENERATIONS.

CELL TYPE - TYPICAL BASAL	Gen 1+2	Gen 1+3	Gen 1+4	Gen 1+5-7	Gen 1+7-16	Gen 2+3	Gen 2+4	Gen 2+5-7	Gen 2+7-16	Gen 3+4	Gen 3+5-7	Gen 3+7-16	Gen 4+5-7	Gen 4+7-16	Gen 5-7 & 7-16
MEASUREMENTS CILIA	0.4825	0.1319	0.2901	0.0162**	0.0044**	0.6085	0.9306	0.2451	0.1187	0.5569	0.3950	0.1599	0.1883	0.0293**	0.4744
APEX OF CELL TO NUCLEUS	0.8382	0.6239	0.1347	0.4686	0.0019**	0.6398	0.3947	0.7169	0.0172**	0.4492	0.8362	0.0090	0.2611	0.0514	0.0085**
APEX OF CELL TO EPITHELIAL BASEMENT MEMBRANE	0.8748	0.4854	0.1268	0.4767	0.0026**	0.5861	0.2597	0.6857	0.0188**	0.4484	0.7914	0.0141**	0.2353	0.0759	0.0069**
CILIA TO EPITHELIAL BASEMENT MEMBRANE	0.8163	0.3188	0.0944	0.3096	0.0010**	0.5432	0.2424	0.5673**	0.0118**	0.4670	0.8989	0.0093**	0.3154	0.0489**	0.0008**
EPITHELIAL BASEMENT MEMBRANE	0.7860	0.6515	0.3187	0.3717	0.0056**	0.9239	0.5039	0.6715	0.0439**	0.5584	0.6556	0.0084**	0.8151	0.0311**	0.0060**

*P VALUES
**SIGNIFICANT p VALUES (p ≤ 0.05)

TABLE 7.1 - D

ANALYSIS OF VARIANCE BETWEEN INDIVIDUAL BRONCHIAL GENERATION

CELL TYPE - ATYPICAL BASAL	Gen 1+2	Gen 1+3	Gen 1+4	Gen 1+5-7	Gen 1+7-16	Gen 2+3	Gen 2+4	Gen 2+5-7	Gen 2+7-16	Gen 3+4	Gen 3+5-7	Gen 3+7-16	Gen 4+5-7	Gen 4+7-16	Gen 5-7 & 7-16
MEASUREMENTS CILIA	0.8476	0.6962	0.6420	0.5090	0.5755	0.5986	0.8695	0.8348	0.8186	0.3446	0.2905	0.2858	0.9507	0.9225	0.9669
APEX OF CELL TO NUCLEUS	0.5909	0.0487**	0.2381	0.0466**	0.0061**	0.2571	0.7152	0.3253	0.1035	0.2635	0.6925	0.6275	0.3228	0.0449**	0.2342
APEX OF CELL TO EPITHELIAL BASEMENT MEMBRANE	0.4636	0.0221**	0.0285**	0.0193**	0.0016**	0.2251	0.3219	0.2947	0.0772	0.6690	0.6669	0.5798	0.9747	0.2222	0.1841
CILIA TO EPITHELIAL BASEMENT MEMBRANE	0.5071	0.0342**	0.0219**	0.0170**	0.0020**	0.2568	0.2483	0.2436	0.0735	0.9184	0.8784	0.4832	0.9568	0.3231	0.2457
EPITHELIAL BASEMENT MEMBRANE	0.0133**	0.1818	0.0176**	0.0267**	0.0023**	0.1489	0.3972	0.2685	0.7124	0.3471	0.4987	0.1830	0.6849	0.4207	0.1823

*P VALUES
**SIGNIFICANT p VALUES ($p \leq 0.05$)

TABLE 7.2A

STATISTICALLY SIGNIFICANT DIFFERENCES OF EPITHELIAL MEASUREMENTS FOR ALL BRONCHIAL GENERATIONS

CILIATED CELLS

CILIA:

GENERATION	1 & 5-7	p =	0.0105
	1 & 7-16	p =	0.0005
	2 & 7-16	p =	0.0134
	3 & 7-16	p =	0.0030
	4 & 7-16	p =	0.0411

APEX TO NUCLEUS:

GENERATION	1 & 3	p =	0.0151
	1 & 4	p =	0.0017
	1 & 5-7	p =	0.0030
	1 & 7-16	p =	0.0000
	2 & 7-16	p =	0.0050
	3 & 7-16	p =	0.0103
	4 & 7-16	p =	0.0407
	5-7 & 7-16	p =	0.0037

BASEMENT MEMBRANE THICKNESS:

GENERATION	1 & 7-16	p =	0.0037
	2 & 7-16	p =	0.0136
	3 & 7-16	p =	0.0045
	4 & 7-16	p =	0.0526
	5-7 & 7-16	p =	0.0007

A (APEX OF CELL TO EPITHELIAL BASEMENT MEMBRANE)

GENERATION	1 & 7-16	p =	0.0026
	2 & 7-16	p =	0.0078
	3 & 7-16	p =	0.0065
	5-7 & 7-16	p =	0.0067

B (A + CILIA)

GENERATION	1 & 7-16	p =	0.0010
	2 & 7-16	p =	0.0051
	3 & 7-16	p =	0.0039
	5-7 & 7-16	p =	0.0059

TABLE 7.2B

STATISTICALLY SIGNIFICANT DIFFERENCES OF EPITHELIAL MEASUREMENTS FOR ALL BRONCHIAL GENERATIONS

NON-CILIATED CELLS

APEX OF CELL TO NUCLEUS:

GENERATION	2 & 5-7	p =	0.0098
	2 & 7-16	p =	0.0033
	3 & 7-16	p =	0.0448
	4 & 7-16	p =	0.0251

BASEMENT MEMBRANE THICKNESS:

NONE ARE SIGNIFICANT

A (APEX OF CELL TO EPITHELIAL BASEMENT MEMBRANE)

GENERATION	1 & 7-16	p =	0.0122
	2 & 5-7	p =	0.0145
	2 & 7-16	p =	0.0016
	3 & 7-16	p =	0.0377
	4 & 7-16	p =	0.0050

B (A + CILIA)

GENERATION	1 & 7-16	p =	0.0122
	2 & 5-7	p =	0.0145
	2 & 7-16	p =	0.0016
	3 & 7-16	p =	0.0377
	4 & 7-16	p =	0.0050

TABLE 7.2C

STATISTICALLY SIGNIFICANT DIFFERENCES OF EPITHELIAL MEASUREMENTS FOR ALL BRONCHIAL GENERATIONS

BASAL CELLS - TYPICAL

CILIA:

GENERATION	1 & 5-7	p =	0.0162
	1 & 7-16	p =	0.0044
	4 & 7-16	p =	0.0293

APEX OF CELL TO NUCLEUS:

GENERATION	1 & 7-16	p =	0.0019
	2 & 7-16	p =	0.0172
	3 & 7-16	p =	0.0090
	5-7 & 7-16	p =	0.0005

BASEMENT MEMBRANE THICKNESS:

GENERATION	1 & 7-16	p =	0.0056
	2 & 7-16	p =	0.0439
	3 & 7-16	p =	0.0084
	4 & 7-16	p =	0.0311
	5-7 & 7-16	p =	0.0060

A (APEX OF CELL TO EPITHELIAL BASEMENT MEMBRANE)

GENERATION	1 & 7-16	p =	0.0026
	2 & 7-16	p =	0.0188
	3 & 7-16	p =	0.0141
	5-7 & 7-16	p =	0.0009

B (A + CILIA)

GENERATION	1 & 7-16	p =	0.0010
	2 & 7-16	p =	0.0118
	3 & 7-16	p =	0.0093
	4 & 7-16	p =	0.0489
	5-7 & 7-16	p =	0.0008

TABLE 7.2D

STATISTICALLY SIGNIFICANT DIFFERENCES OF EPITHELIAL MEASUREMENTS FOR ALL BRONCHIAL GENERATIONS

BASAL CELLS - ATYPICAL

CILIA:

NONE ARE SIGNIFICANT

APEX OF CELL TO NUCLEUS:

GENERATION	1 & 3	p =	0.0487
	1 & 5-7	p =	0.0464
	1 & 7-16	p =	0.0061
	4 & 7-16	p =	0.0449

BASEMENT MEMBRANE THICKNESS:

GENERATION	1 & 2	p =	0.0133
	1 & 4	p =	0.0176
	1 & 5-7	p =	0.0267
	1 & 7-16	p =	0.0023

A (APEX OF CELL TO EPITHELIAL BASEMENT MEMBRANE)

GENERATION	1 & 3	p =	0.0221
	1 & 4	p =	0.0285
	1 & 5-7	p =	0.0193
	1 & 7-16	p =	0.0016

B (A + CILIA)

GENERATION	1 & 3	p =	0.0342
	1 & 4	p =	0.0219
	1 & 5-7	p =	0.0170
	1 & 7-16	p =	0.0020

TABLE 8A:

BASAL CELL POPULATION NON-SMOKERS ENUMERATION BY AIRWAY GENERATION

Airway Generation	1	2	3	4	5-7	7-16
Diameter (mm)	> 9.01	7.0 - 9	5.51 - 7	4.51 - 5	2.01 - 4.5	0.26 - 2
Typical Cells	*416	496	389	316.5 ± 88.5	195.7 ± 22.8	57.3 ± 3.7
Atypical Cells	161.7	23	68	50.9 ± 38.2	46.8 ± 11.7	8.5 ± 6.4
% Typical Cells	72	95.6	85.1	86.1	80.7	87.2
% Atypical Cells	28	4.4	14.9	13.9	19.3	12.8

* Mean ± Standard Error

' ABSENCE OF STANDARD ERROR IS DUE TO INSUFFICIENT NUMBER OF CASES

TABLE: 8B

BASAL CELL POPULATION SMOKERS ENUMERATION BY AIRWAY GENERATION

Airway Generation	1	2	3	4	5-7	7-16
Diameter (mm)	> 9.01	7.0 - 9	5.51 - 7	4.51 - 5	2.01 - 4.5	0.26 - 2
Typical Cells	*495.2 ± 97.5	377.9 ± 116.7	349.6 ± 61.8	355.4 ± 67.5	253.1 ± 37.1	79.3 + 9.3
Atypical Cells	79.0 ± 18.2	87.8 ± 38.8	73.6 ± 11.2	61.4 ± 9.7	53.8 ± 7	23.9 + 4.3
% Typical Cells	86.2	81.1	82.6	85.3	82.5	76.8
% Atypical Cells	13.8	18.9	17.4	14.7	17.5	23.2

* Mean ± Standard Error.

[1] ABSENCE OF STANDARD ERROR IS DUE TO INSUFFICIENT NUMBER OF CASES

TABLE 9A

EPITHELIAL MEASUREMENTS (μm) WITH RESPECT TO ANATOMICAL LOCATION
SMOKERS AND NON-SMOKERS COMBINED

AIRWAY GENERATION: 1

AIRWAY DIAMETER: ≥9.01 mm

	RIGHT UPPER LOBE * n=0	RIGHT LOWER LOBE n=3	LEFT UPPER LOBE n=2	LEFT LOWER LOBE n=3	P VALUE
CILIA	-	5.88 ± 0.48	5.75 ± 0.52	7.15 ± 0.31	0.1204
APEX OF CELL TO EPITHELIAL BASEMENT MEMBRANE	-	15.72 ± 7.88	16.25 ± 16.25	34.10 ± 5.94	0.3306
CILIA TO EPITHELIAL BASEMENT MEMBRANE	-	19.63 ± 7.29	19.13 ± 13.90	27.19 ± 7.74	0.7766
BASEMENT MEMBRANE THICKNESS	-	4.62 ± 0.45	4.47 ± 1.95	4.28 ± 0.66	0.9629

* NUMBER OF LOBES MEASURED
NUMBERS REPRESENT MEANS ± STANDARD ERROR

TABLE 9B

EPITHELIAL MEASUREMENTS (μm) WITH RESPECT TO ANATOMICAL LOCATION
SMOKERS AND NON-SMOKERS COMBINED

AIRWAY GENERATION: 2

AIRWAY DIAMETER: 7.01 - 9.00 mm

	RIGHT UPPER LOBE	RIGHT LOWER LOBE	LEFT UPPER LOBE	LEFT LOWER LOBE	P VALUE
	* n=0	n=1	n=1	n=1	
CILIA	-	5.05	7.00	6.92	**
APEX OF CELL TO EPITHELIAL BASEMENT MEMBRANE	-	40.74	38.79	32.04	"
CILIA TO EPITHELIAL BASEMENT MEMBRANE	-	29.22	42.92	34.60	"
BASEMENT MEMBRANE THICKNESS	-	4.38	4.95	6.10	"

* NUMBER OF LOBES MEASURED
** INSUFFICIENT DATA FOR ANALYSIS
NUMBERS REPRESENT MEANS + STANDARD ERROR

TABLE 9C

EPITHELIAL MEASUREMENTS (µm) WITH RESPECT TO ANATOMICAL LOCATION
SMOKERS AND NON-SMOKERS COMBINED

AIRWAY GENERATION: 3

AIRWAY DIAMETER: 5.51 - 7.00 mm

	RIGHT UPPER LOBE * n=3	RIGHT LOWER LOBE n=2	LEFT UPPER LOBE n=1	LEFT LOWER LOBE n=3	P VALUE
CILIA	5.22 ± 0.59	5.79 ± 0.29	5.91	6.75 ± 0.29	0.2070
APEX OF CELL TO EPITHELIAL BASEMENT MEMBRANE	9.43 ± 9.43	26.71 ± 2.31	40.51	39.22 ± 7.20	0.1319
CILIA TO EPITHELIAL BASEMENT MEMBRANE	14.14 ± 8.52	24.11 ± 4.26	32.56	33.87 ± 8.68	0.4085
BASEMENT MEMBRANE THICKNESS	5.12 ± 0.72	4.99 ± 2.20	5.65	3.95 ± 0.31	0.7541

* NUMBER OF LOBES MEASURED
NUMBERS REPRESENT MEANS ± STANDARD ERROR

TABLE 9D

EPITHELIAL MEASUREMENTS (μm) WITH RESPECT TO ANATOMICAL LOCATION
SMOKERS AND NON-SMOKERS COMBINED

AIRWAY GENERATION: 4

AIRWAY DIAMETER: 4.51 - 5.50 mm

	RIGHT UPPER LOBE * n=2	RIGHT LOWER LOBE n=2	LEFT UPPER LOBE n=3	LEFT LOWER LOBE n=4	P VALUE
CILIA	5.61 ± 0.19	5.83 ± 0.02	6.30 ± 0.48	5.98 ± 0.30	0.6462
APEX OF CELL TO EPITHELIAL BASEMENT MEMBRANE	29.31 ± 0.97	23.90 ± 0.87	32.39 ± 7.31	32.19 ± 6.76	0.8263
CILIA TO EPITHELIAL BASEMENT MEMBRANE	31.98 ± 1.10	24.04 ± 1.18	33.02 ± 4.37	33.18 ± 7.98	0.7964
BASEMENT MEMBRANE THICKNESS	4.21 ± 0.18	3.85 ± 0.22	4.38 ± 0.88	4.64 ± 0.81	0.9176

* NUMBER OF LOBES MEASURED
NUMBERS REPRESENT MEANS ± STANDARD ERROR

TABLE 9E

EPITHELIAL MEASUREMENTS (μm) WITH RESPECT TO ANATOMICAL LOCATION
SMOKERS AND NON-SMOKERS COMBINED

AIRWAY GENERATION: 5 - 7

AIRWAY DIAMETER: 2.01 - 4.50 mm

	RIGHT UPPER LOBE n=4	RIGHT LOWER LOBE n=5	LEFT UPPER LOBE n=5	LEFT LOWER LOBE n=5	P VALUE
CILIA	6.05 ± 0.50	5.78 ± 0.26	6.11 ± 0.36	5.92 ± 0.09	0.8777
APEX OF CELL TO EPITHELIAL BASEMENT MEMBRANE	17.82 ± 6.21	27.44 ± 5.99	35.44 ± 4.19	31.23 ± 3.58	0.1468
CILIA TO EPITHELIAL BASEMENT MEMBRANE	21.60 ± 5.25	28.57 ± 4.41	32.39 ± 1.96	30.40 ± 3.30	0.2810
BASEMENT MEMBRANE THICKNESS	4.37 ± 0.09	5.11 ± 0.56	4.45 ± 0.53	4.55 ± 0.51	0.7118

* NUMBER OF LOBES MEASURED
NUMBERS REPRESENT MEANS ± STANDARD ERROR

TABLE 9F

EPITHELIAL MEASUREMENTS (μm) WITH RESPECT TO ANATOMICAL LOCATION
SMOKERS AND NON-SMOKERS COMBINED

AIRWAY GENERATION: 7 - 16

AIRWAY DIAMETER: 0.26 - 2.00 mm

	RIGHT UPPER LOBE * n=3	RIGHT LOWER LOBE n=4	LEFT UPPER LOBE n=4	LEFT LOWER LOBE n=6	P VALUE
CILIA	8.56 ± 3.59 **	5.50 ± 0.31	5.37 ± 0.35	5.47 ± 0.23	0.3258
APEX OF CELL TO EPITHELIAL BASEMENT MEMBRANE	27.54 ± 6.59	23.55 ± 5.94	24.87 ± 3.43	18.96 ± 2.18	0.5245
CILIA TO EPITHELIAL BASEMENT MEMBRANE	23.59 ± 1.21	25.73 ± 3.80	26.48 ± 3.74	23.41 ± 2.18	0.8459
BASEMENT MEMBRANE THICKNESS	3.73 ± 0.42	2.94 ± 0.52	3.24 ± 0.60	3.00 ± 0.52	0.7883

* NUMBER OF LOBES MEASURED
NUMBERS REPRESENT MEANS ± STANDARD ERROR

TABLE: 10

MEASUREMENTS OF DUST-LADEN MACROPHAGES TO BASAL CELL NUCLEI (μm)

	Gen. 1	Gen. 2	Gen. 3	Gen. 4	Gen. 5-7	Gen. 7-16	P VALUE
TYPICAL BASAL CELLS. MACROPHAGE TO BASAL CELL NUCLEUS	-	-	-	4.11 ± 4.11	7.14 ± 2.73	9.03 ± 3.70	0.3145
ATYPICAL BASAL CELLS. MACROPHAGE TO BASAL CELL NUCLEUS	-	-	-	3.43 ± 3.43	5.45 ± 3.13	4.00 ± 2.54	0.8008

NUMBERS REPRESENT MEANS ± STANDARD ERROR

TABLE: 11

HYPERPLASIA AND METAPLASIA MEASUREMENTS (μm) - SMOKERS

	Gen. 1	Gen. 2	Gen. 3	Gen. 4	Gen. 5-7	Gen. 7-16
HYPERPLASIA	43.95 ± 3.61	47.49 ± 1.66	45.92 ± 5.84	43.45 ± 8.97	43.02 ± 2.59	37.08 ± 4.39
METAPLASIA	35.01 ± 6.56	24.14	27.56 ± 4.36	35.47 ± 6.92	29.97 ± 3.37	49.83 ± 31.00

NUMBERS REPRESENT MEANS ± STANDARD ERROR

F. REFERENCES

Auerbach, O. Natural History of Carcinoma of the Lung. Pulmonary Diseases and Disorders. Ed. A. P. Fishman., McGraw-Hill Book Co., New York., 1980, p. 1388.

Ayers, W., and Jeffrey, P.R. Cellular Pathology of the Lung. Ed. G. Cummings and G. Bonsignor., Plenum Press, Inc., New York, 1982., p. 33

Becci, P.J., McDowell, E.M., and Trump, B.F. The Respiratory Epithelium II Hamster Trachea, Bronchus and Bronchioles. J. Natl. Cancer Inst. 61: 551-561, 1978

Bindreiter, M., S[illegible]pler, J., and Stochinger L. Zellproliferation and Differenzierung im Tracheal Epithel Der Ratte. Exp. Cell Res. 50: 377, 1968.

Boren, H.G. and Paradise, L.J. Cytokinetics of the Lung in Pathogenesis and Therapy of Lung Cancer. (Ed. C.C. Harris), Marcel Dekher, New York. pp 369 - 418, 1978.

Bowden, D.H. Cell Turnover in the Lung. Am Rev. Respir. Dis. 128: S46-S48, 1983.

Bowden, D.H. Macrophages, Dust and Pulmonary Diseases. Exp. Lung Res. 12: 89-107, 1987.

Committee on the Biological Effects of Ionizing Radiations, et al. Health Risks of Radon and Other Internally Deposited Alpha-Emitters. **(BEIR III)**, National Academy Press. Washington, D.C., 1980.

Committee on the Biological Effects of Ionizing Radiations, et al. Health Risks of Radon and Other Internally Deposited Alpha-Emitters. **(BEIR IV)**, National Academy Press. Washington, D.C., 1988.

Donnelly,G.M., Haack, D.G.,and Heird, C.S. Tracheal Epithelium: Cell Kinetics and Differentiation in Normal Rat Rissue. Cell Tissue Kinet. 15: 119-130, 1982.

Evans, M.J. Shami, S.G., Cabral-Anderson, L.J. and Dekker, N.P. Role of Non-Ciliated Cells in Renewal of the Bronchial Epithelium of Rats Exposed to NO_2. Am. J. Pathol. 123: 126-133, 1986.

Evans, M.J., Plopper, C.G. Personal Communication, 1987.

Gastineau, R.M., Walsh, P.J. and Underwood, N. Thickness of Bronchial Epithelium with Relation to Exposure to Radon. Health Physics. 23: 857-860, 1972.

Gehr,P., Bachofen, M., and Weibel, E.R. The Normal Human Lung: Ultrastructue and Morphometric Estimation of Diffusion Capacity. Resp. Physiol. 32: 121, 1978.

Inayama, Y., Hoog, G.E.R., Brody, A.R., Cameron, G.S., Jetten, A.M., Gilmore, L.M., Gray, T., and Nettersheim, P. The Differentiation Potential of Tracheal Basal Cells. Lab Invest. 58: 706-717, 1988.

International Commission on Radiological Protection, Publication 50. Lung Cancer Risk from Indoor Exposures to Radon Daughters. Annals of the ICRP. Pergamon Press. Oxford. 7:1, 1987

Jeffrey, P.K. and Reid, L. New Observations of Rat Airway Epithelium. A quantitative and Electron Microscopic Study. J. Anat. 120: 295-329, 1975.

Kawanami, D., Ferranus, V.J. and Crystal, R.G. Anchoring Fibrils in the Normal Canine Respiratory System. Am. Rev. Resp. Dis. 120: 586-611, 1979.

Keenan, K.P., Combs, J.W., and McDowell, E.M. Regeneration of Hamster Tracheal Epithelium after Mechanical Injury. I. Focal Lesions: Quantitative Morphological Study of Cell Proliferation. Virchows Arch. (B) 41: 193-214., 1982a.

Keenan, K.P., Combs, J.W., and McDowell, E.M. Regeneration of Hamster Tracheal Epithelium after Mechanical Injury. II. Multifocal Lesions: Stathmokinetic and Autoradiographic Studies of Cell Proliferation. Virchows Arch. (B) 41: 215-229., 1982b.

Kilburn, K.H. An Hypotheses of Pulmonary Clearance and its Implications. Am. Rev. Respir. Dis. 98: 449, 1968.

Lane, B.P. and Gordon, R. Degeneration of Rat tracheal Epithelium after Mechanical Injury. I. The Relationship Between Mitotic Activity and Cellular Differentiation., Proc. Soc. Exp. Biol. Med. 145: 1139-1144, 1974.

Lea, D.E.A. Action of Radiation on Living Cells. 2nd Edn. Cambridge University Press, Cambridge. 1955.

Lentz, T.L. In: Cell Fine Structure, W.B. Saunders. Philadelphia, 1971.

McDowell, E.M., Barrett, L.A., Glavin, F., Harris, C.C. and Trump, B.F. The Respiratory Epithelium. (1) Human Bronchus. J. Nat. Can. Inst. 61: 533-549, 1978.

McDowell, E.M., Keenan, K.P., and Huang, M. Restoration of Mucociliary Tracheal Epithelium Following Deprivation of Vitamin A. Virchows Arc. 45: 221-240, 1984.

McDowell, E.M., Newkirk, C. and Coleman, B. Development of Hamster Tracheal Epithelium. II. Cell Proliferation in the Fetus. Anat. Rec. 213: 448-456, 1985.

Matsuba, K. and Thurlbeck, W.M. Disease of the Small Airways in Chronic Bronchitis. Am. Rev. Respir. Dis. 107: 551-558, 1973.

Plopper, C.G. , Mariassy, A.T., Wilson, D.W., Abbey, J.L., Nishio, S.T. and Nettesheim, P. Comparison of Non-Cililated Tracheal Epithelial Cells in Six Mammalian Species: Ultrastructural and Population Densities. Exp. Lung. Res. 5: 281-298, 1983.

Reid, L. and Jones, R. Bronchial Mucosal Cells. Fed. Proc. 38: 191-196, 1979.

Sevc, J., Kunz, E. and Tomasek, L., Cancer in Man After Exposure to Rn Daughters. Health Physics., 54: 27-46, 1988.

Spencer, H., Anatomy of the Lung. Pathology of the Lung. Pergamon Press Ltd., Oxford. 4th Edition, 1985, p. 17.

Thurlbeck, W.M. Chronic Airflow Obstruction in Lung Disease. W.B. Saunders, Philadelphia, 1976.

Till, J.E., McCullough, E.A. Hemopoietic Stem Cell Differentiation. Biochem. Biophys. Acta. 605: 431-459, 1980.

Trembath, P.W. Bronchial Mucus and Mucociliary Transport. In: Respiratory Medicine. Scadding, J.G., Cumming, G. and Thurlbeck, W.M. (Eds), W.M. Heinemann, London, 1981.

van Furth, R. The Origin and Turnover of Promonocytes, Monocytes and Macrophages in Normal Mice. In: Mononuclear Phagocytes. van Furth. (Ed), Blackwell, Oxford., pp 151-165, 1970.

Volkman, A. Disparity in Origin of Mononuclear Phagocyte Populations. J. Reticuloendothel. Soc. 19: 249-268, 1976.

Weibel, E.R. Morpohometry of the Human Lung. Springer Verlag, Heidelberg; and Academic Press, New York, 1963.

Weiss, D.L. and Tweedale, D.N. Inflation Fixation of Lungs: Use of a Simple, Inexspensive Apparatus. Amer. rev. Resp. Dis. 94: 629-631, 1966.

Woodworth, C.D., Mossman, B.T. and Craighead, J.E. Interaction of Asbestos with Metaplastic Squamous Epithelium Developing in Organ Cultures. Environ. Health Perspect. 51: 27-33, 1983.

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H. APPENDICES

THE BRONCHIAL TREE — APPENDIX A

The model of the bronchial tree (Weibel, 1963), (Figure 1), which is in general use assumes that airways branch by symmetrical dichotomy. However, a number of assumptions, were made in the calculations which did not, take into account the asymmetry of the bronchial tree.

From the Weibel model (1963) of symmetrical dichotomy it is evident that the number of branches increase with each ascending generation. The caluclations include the diameter and the length of each generation of branches. The volume of the lung has been calculated to be 4,341 ml + 285 ml (Gehr et al, 1978). Using the Weibel (1963) data for diameter, length and the number of branches of each generation, the proportion of the lung volume occupied by each generation was calculated as follows:

- area of cross-section of bronchus = r^2
- volume of bronchus area of cross-section x length
- total volume of bronchi of each generation = volume of bronchus x number of branches

The total lung volume occupied by each generation of bronchus was expressed as a percentage (Figure A-2).

From these calculations, it is evident that the different generations of the bronchial tree occupy only a small proportion of the total lung volume and, it is not suprising therefore that under the microscope, the presence of more than one bronchial branch in a section was the exception rather than the rule. Even the most numerous of conducting airways (generations 14, 15 and 16) were rarely present in the same section. From examining all sections of the study, it was found that when two bronchial branches were in close proximity, the distance betwen the lumen of one bronchus and the cytoplasm of the other was rarely less than 0.35 mm (Figure A-3). Thus, it appears that even for bronchi which are the most numerous in the lung, the distances between conducting airways are too great for transmission of energy from a source in one lumen of a bronchus to the nuclei of the epithelium of another, with the possible exception of sites at branching. However, tissue in the interstitum between adjacent bronchi could be within range.

Number of branches	*Generation*	*Diameter (mm)*	*Length (mm)*	*Structure*
1	0	18·0	120·0	trachea
2	1	12·2	47·6	main bronchi
4	2	8·3	19·0	
8	3	5·6	7·6	
16	4	4·5	12·7	
32	5	3·5	10·7	
64	6	2·8	9·0	
128	7	2·3	7·6	
256	8	1·86	6·4	
512	9	1·54	5·4	
1024	10	1·30	4·6	
2048	11	1·09	3·9	
4096	12	0·95	3·3	
8192	13	0·82	2·7	
16384	14	0·74	2·3	
32768	15	0·66	2·0	
65536	16	0·60	1·65	TB
131072	17	0·54	1·41	RB
262144	18	0·50	1·17	RB
524288	19	0·47	0·99	RB
1048576	20	0·45	0·83	AD
2097152	21	0·43	0·70	AD
4194304	22	0·41	0·59	AD
8388608	23	0·41	0·50	AD

TB = terminal bronchiole; RB = respiratory bronchiole; AD = alveolar duct.

FIGURE A - 1 Model of the Bronchial Tree.
(Weibel, 1963)

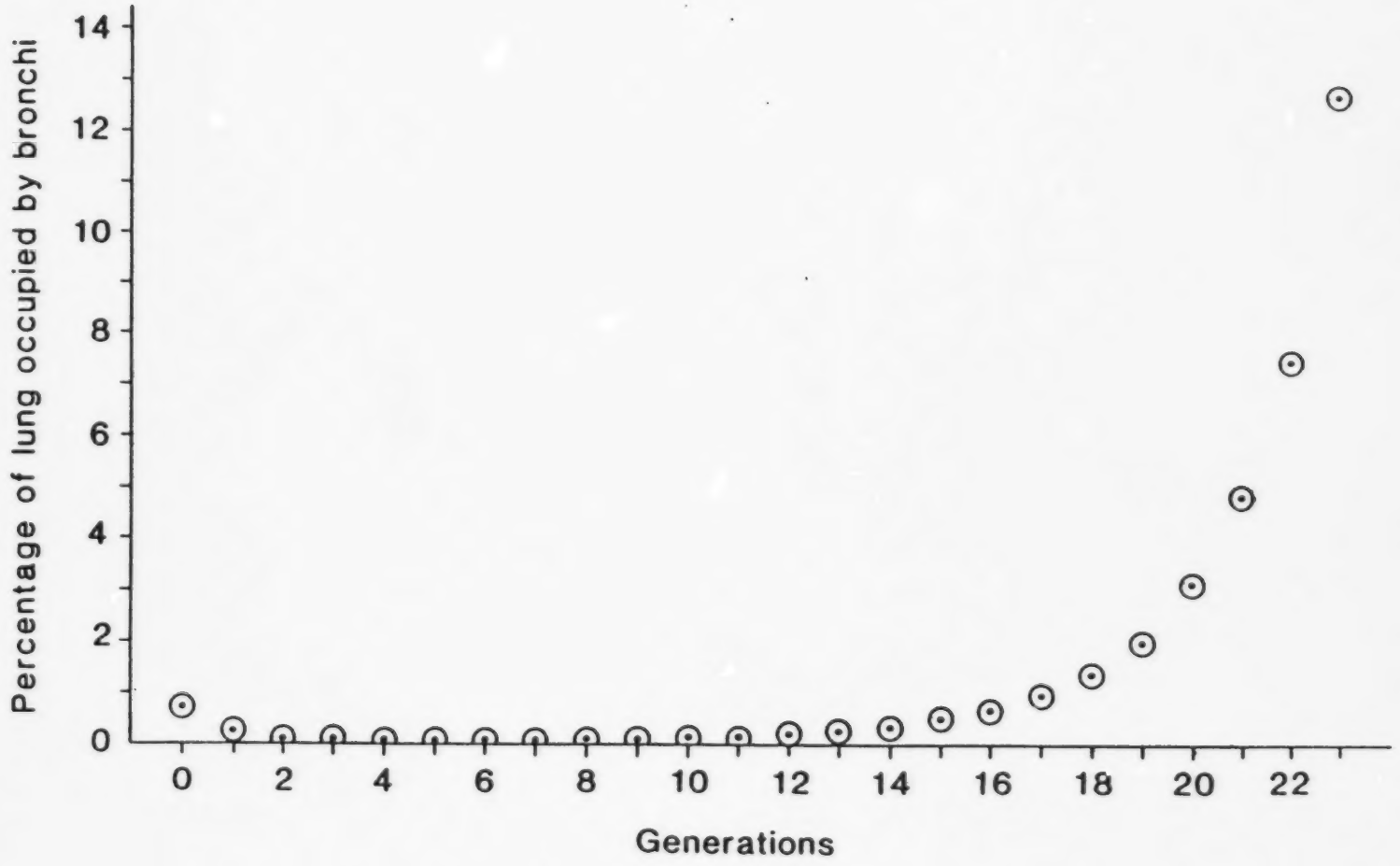

FIGURE A-2 Volume of Lung Occupied by Bronchi.

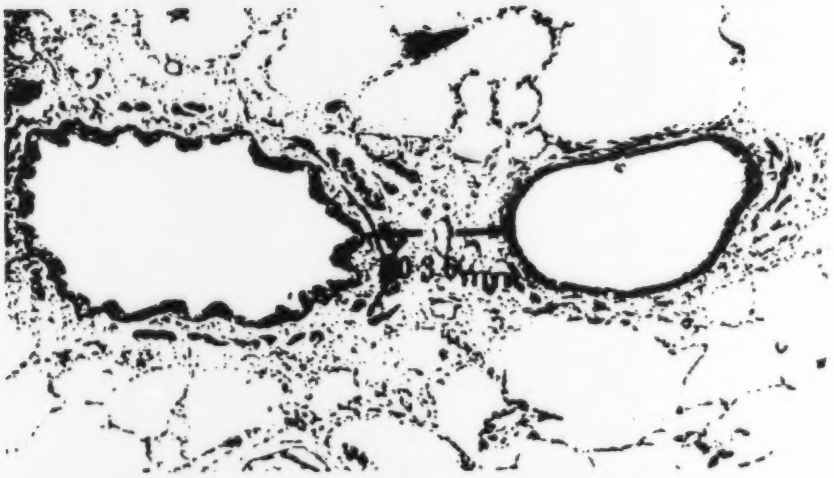

FIGURE A-3 Bronchi in Close Proximity. x 60